Biochemical and Biophysical Research Communications 436 (2013) 418–423

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Haojie Hao [a], Jiejie Liu [a], Jing Shen [b], Yali Zhao [a], Huilin Liu [a], Qian Hou [a], Chuan Tong [a], Dongdong Ti [a], Liang Dong [a], Yu Cheng [b], Yiming Mu [b], Jianping Liu [b,c], Xiaobing Fu [a,*], Weidong Han [a,*]

[a] Institute of Basic Medicine Science, College of Life Science, Chinese PLA General Hospital, Beijing 100853, China
[b] Endocrinology of Department, Chinese PLA General Hospital, Beijing 100853, China
[c] Department of Endocrinology, Jiangxi Provincial People's Hospital, Nanchang, Jiangxi Province, China

ARTICLE INFO

Article history:
Received 12 May 2013
Available online 11 June 2013

Keywords:
Bone marrow
Mesenchymal stem cells
Type 2 diabetes
Multiple infusion
Hyperglycemia

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.

* Corresponding authors. Address: Institute of Basic Medicine Science, College of Life Science, Chinese PLA General Hospital, No. 28 Fuxing Road, Beijing 100853, China. Fax: +86 10 66937516.

E-mail addresses: fuxiaobing@vip.sina.com (X. Fu), hanwdrsw69@yahoo.com (W. Han).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.117

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.

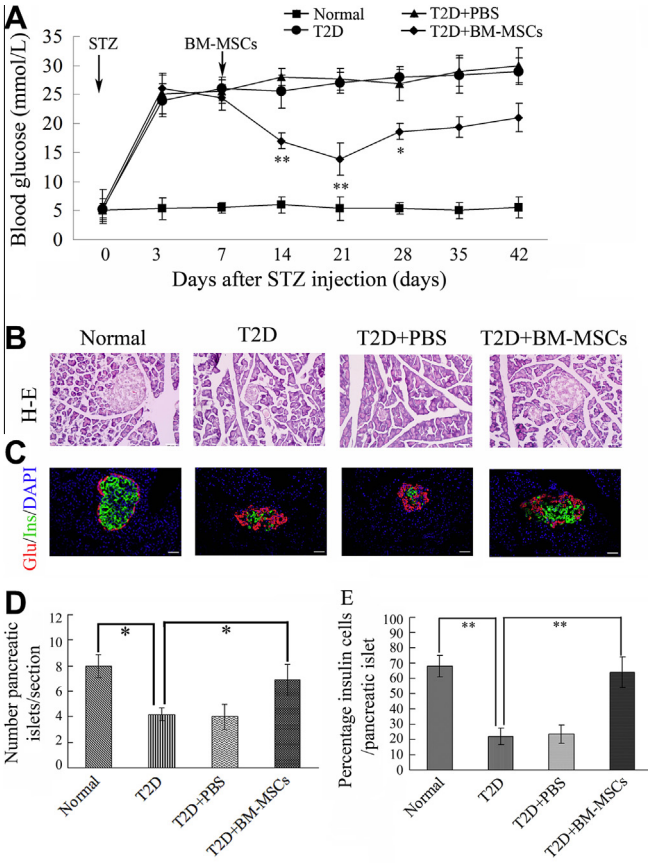

Fig. 1. Single-dose BM-MSC infusion ameliorated hyperglycemia in T2D rats. (A) Blood glucose levels were determined consecutively in fasted rats. (B) Morphology of pancreatic islets stained with H–E. (C) Pancreatic islets were characterized by immunofluorescence according to the presence and distribution 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 $^{**}P < 0.01$).

2.6. Histopathology and immunofluorescence staining

Pancreas histology was examined after harvesting and formalin-fixation of the pancreas, followed by paraffin-embedding. Sections were cut at 5 μm and stained with hematoxylin–eosin (H–E). Immunofluorescence staining was also performed. Sections were incubated with anti-mouse insulin (1:400, Sigma–Aldrich, St. Louis, MO, USA), rabbit anti-rat glucagon (1:200, Sigma–Aldrich) at 4 °C for overnight. The sections were then washed with PBS with Tween 20 and incubated with Alexa Fluor 594-conjugated anti-mouse IgG (1:1000, Invitrogen, Grand Island, NY, USA) and Alexa Fluor 488-conjugated anti-rabbit IgG (1:1000, Invitrogen) for 1 h and then stained with DAPI (Vector, Burlingame, CA, USA) at 0.1 mg/ml. Sections were observed by fluorescence microscopy (Leica DM5000B, Germany) with 420- and 590-nm filters. Images were analyzed by using the Leica QWin system.

2.7. Statistical analysis

All data are presented as mean ± SE and represent an average of at least triplicate samples. Statistical analyses were performed using Student's *t*-tests for paired samples. The statistical significance was accepted for *P* values of <0.05.

3. Results

3.1. Single-dose BM-MSC infusion ameliorated hyperglycemia and promoted the recovery of T2D pancreas damage in T2D rats induced by high-fat diet/STZ

The effect of BM-MSC infusion was tested in a T2D rat model established by a high-fat diet and STZ administration. The model was confirmed by checking blood glucose, OGTTs, and IPITTs. On day seven after STZ injection, blood glucose levels in the STZ-induced group increased by 24 ± 2.3 mmol/L. The consequences of OGTTs showed a significant deterioration in glucose metabolism, and IPITTs revealed reduced insulin sensitivity (Supplementary Fig. 1A–C). Moreover, most rats showed typical clinical symptoms of diabetes including polyphagia, polydipsia and polyuria.

At day seven, after STZ injection, a single dose of BM-MSCs (1×10^6 cells) was transplanted via intravenous injection into T2D rats, and blood glucose levels were monitored (Fig. 1A). By

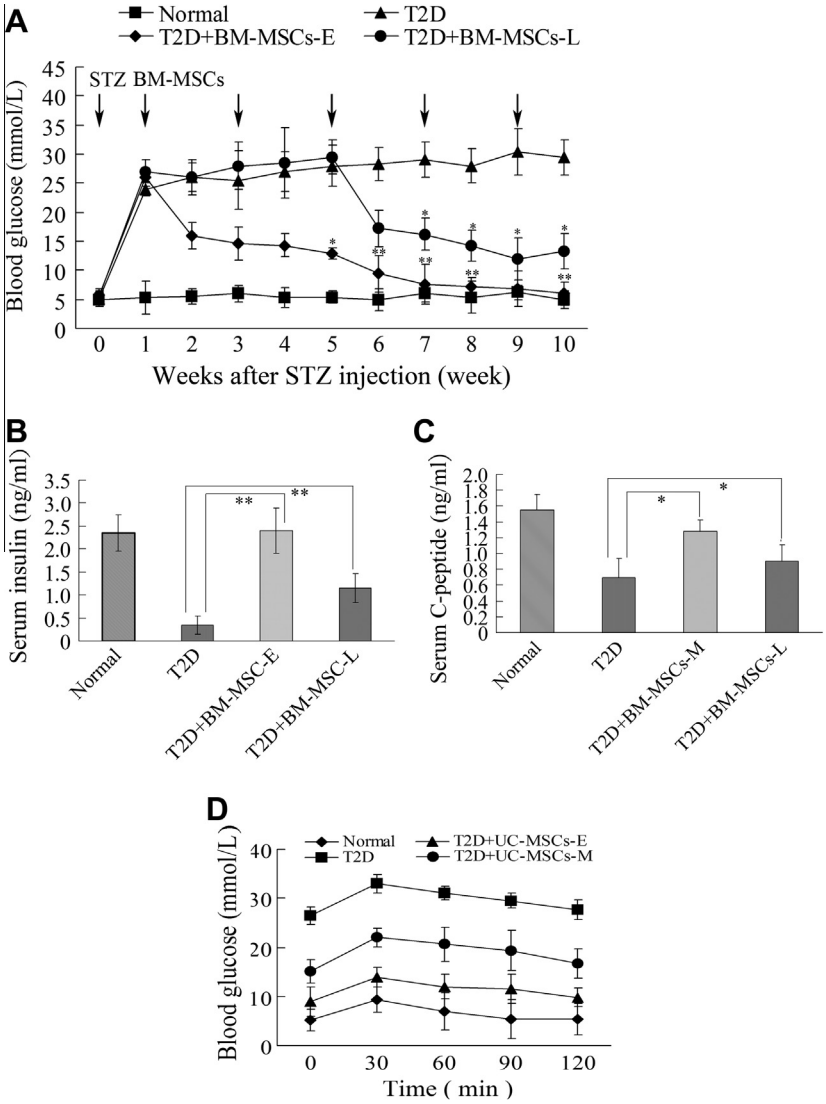

Fig. 2. Multiple BM-MSC infusions in T2D rats. (A) Blood glucose levels were determined consecutively in fasting rats. (B) Individual insulin levels in fasting and re-fed rats were evaluated by ELISA (n = 10 rats per group). (C) Individual C-peptide levels in fasted and re-fed rats were evaluated by ELISA (n = 10 rats per group). (D) Individual oral glucose tolerance was assessed by OGTTs and determining blood glucose levels 9 weeks after MSC infusion. Data are shown as mean ± SD (*P < 0.05 and **P < 0.01).

day seven after treatment, blood glucose levels were reduced in T2D rats administered BM-MSCs, and levels reached a minimum at day 14. Blood glucose levels then increased gradually, but were still no higher than 21 ± 3.4 mmol/L at day 35. In contrast, untreated T2D rats and control rats treated with PBS remained severely hyperglycemic. Histological analysis showed morphological destruction of pancreatic islets in T2D rats and T2D+PBS rats, characterized by a significant reduction in islet size. The islets appeared larger in pancreases from T2D rats are treated with one of BM-MSCs, but were still smaller than in normal rats (Fig. 1B). Immunofluorescence staining showed that BM-MSC infusion ameliorated the morphological and structural damage to pancreatic islets (Fig. 1C), and led to significant restoration of the ratio of insulin-positive cells per islet (Fig. 1D). The numbers of pancreatic islets were also substantially increased, but remained below normal levels (Fig. 1E).

These results demonstrated that a single dose of MSCs could ameliorate hyperglycemia and relieve STZ-induced pancreatic damage in T2D rats, though the effect was only maintained for 2–3 weeks, and normoglycemia was not restored. These observations were consistent with a previous report indicating that a single intravenous dose of MSCs diminished hyperglycemia only transiently in STZ-induced diabetic animal models [6].

3.2. Multiple intravenous BM-MSC infusions stabilized blood glucose

Single-dose BM-MSC infusion ameliorated hyperglycemia and prevented further raises in blood glucose levels but failed to restore normoglycemia in T2D rats. We therefore investigated the effects of multiple BM-MSC infusions in T2D rats. Based on the results of single-dose transfusions, serially BM-MSC infusions were administered to T2D rats via the tail vein once every 2 weeks. Blood glucose levels gradually decreased after three infusions administered to early-phase (1 week) or late-phase (5 week) T2D rats. Furthermore, blood glucose levels reached almost normal levels in early-phase T2D rats after five MSC infusions, while control T2D rats and T2D+PBS group rats remained hyperglycemic (Fig. 2A). Serum insulin and C-peptide levels were significantly increased by multiple BM-MSC infusions (Fig. 2B and C). OGTTs indicated that glucose metabolism was significantly ameliorated by multiple BM-MSC infusions (Fig. 2D). These data suggested that multiple BM-MSC infusions can ameliorate hyperglycemia in T2D rats. Blood glucose levels were restored to near normal in T2D rats, and normal blood glucose levels were maintained for at least 9 weeks.

3.3. Multiple intravenous BM-MSC infusions restored damaged pancreatic islets

We evaluated the effects of BM-MSC transfusion on the recovery of the damaged pancreatic islets in T2D rats by double-labeled immunofluorescence staining for insulin/glucagon. The results showed that damaged pancreatic islets gradually recovered to near normal (Fig. 3A) after multiple BM-MSC infusions, while the number of pancreatic islets and the ratio of insulin-positive cells per islet were likewise restored to almost normal levels (Fig. 3B and C). Late-phase T2D rats only received three BM-MSC infusions, which failed to reverse hyperglycemia or restore normal pancreatic islets. These data suggested that multiple BM-MSC infusions could be restored the damaged pancreatic islets to near normal in T2D rats.

3.4. BM-MSC CM ameliorated hyperglycemia in T2D rats

We further investigated whether MSC-secreted paracrine factors ameliorated hyperglycemia in T2D rats. Serially BM-MSC CM infusions were administered to T2D rats via the tail vein once every 3 days. Continuous blood glucose assays showed that CM considerably ameliorated hyperglycemia in T2D rats. Blood glucose levels

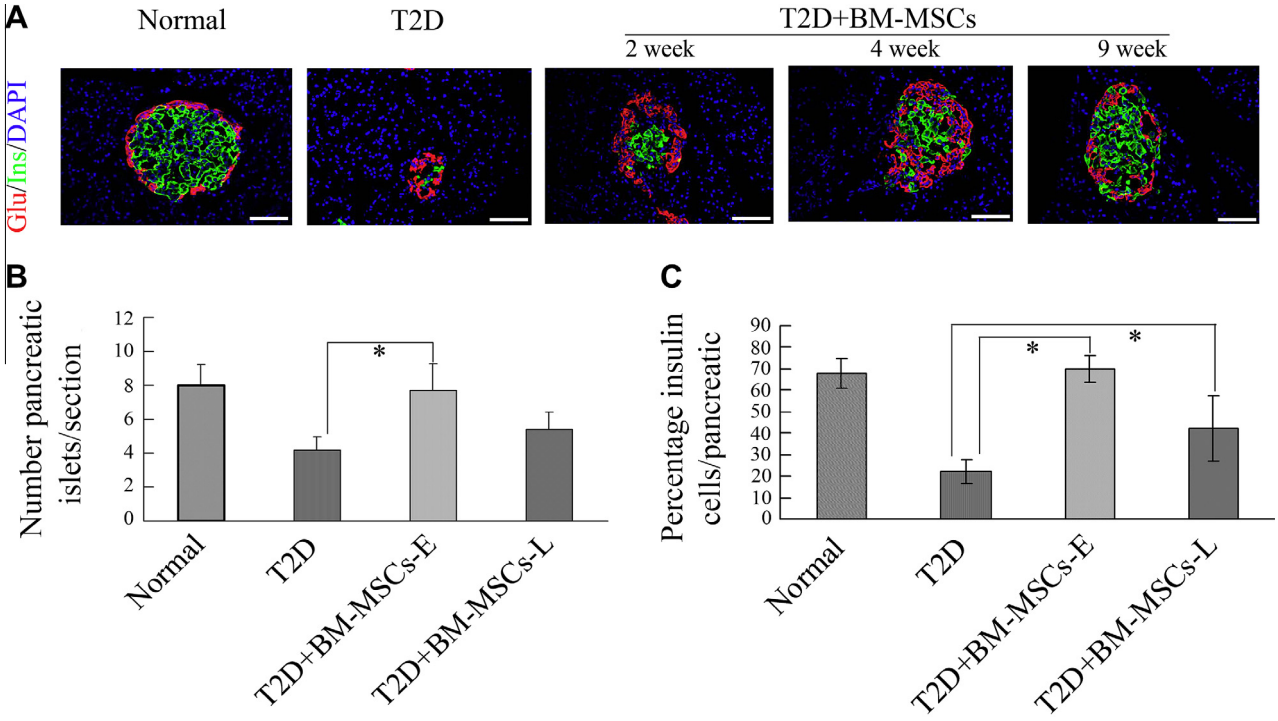

Fig. 3. Damaged pancreatic islets were restored by multiple BM-MSC infusions. (A) Pancreatic islets were characterized by immunofluorescence according to the presence and distribution of insulin- (red) and glucagon-producing (green) cells. (B) Pancreatic islets observed in immunofluorescence-stained sections were quantified in T2D rats administered MSC infusions ($n = 5$ sections per group). (G) β-Cells in pancreatic islets were quantified in T2D and MSC-treated T2D rats administered MSC infusions ($n = 5$ sections per group). Scale bar: 50 μm. Data are shown as mean ± SD ($^{*}P < 0.05$ and $^{**}P < 0.01$).

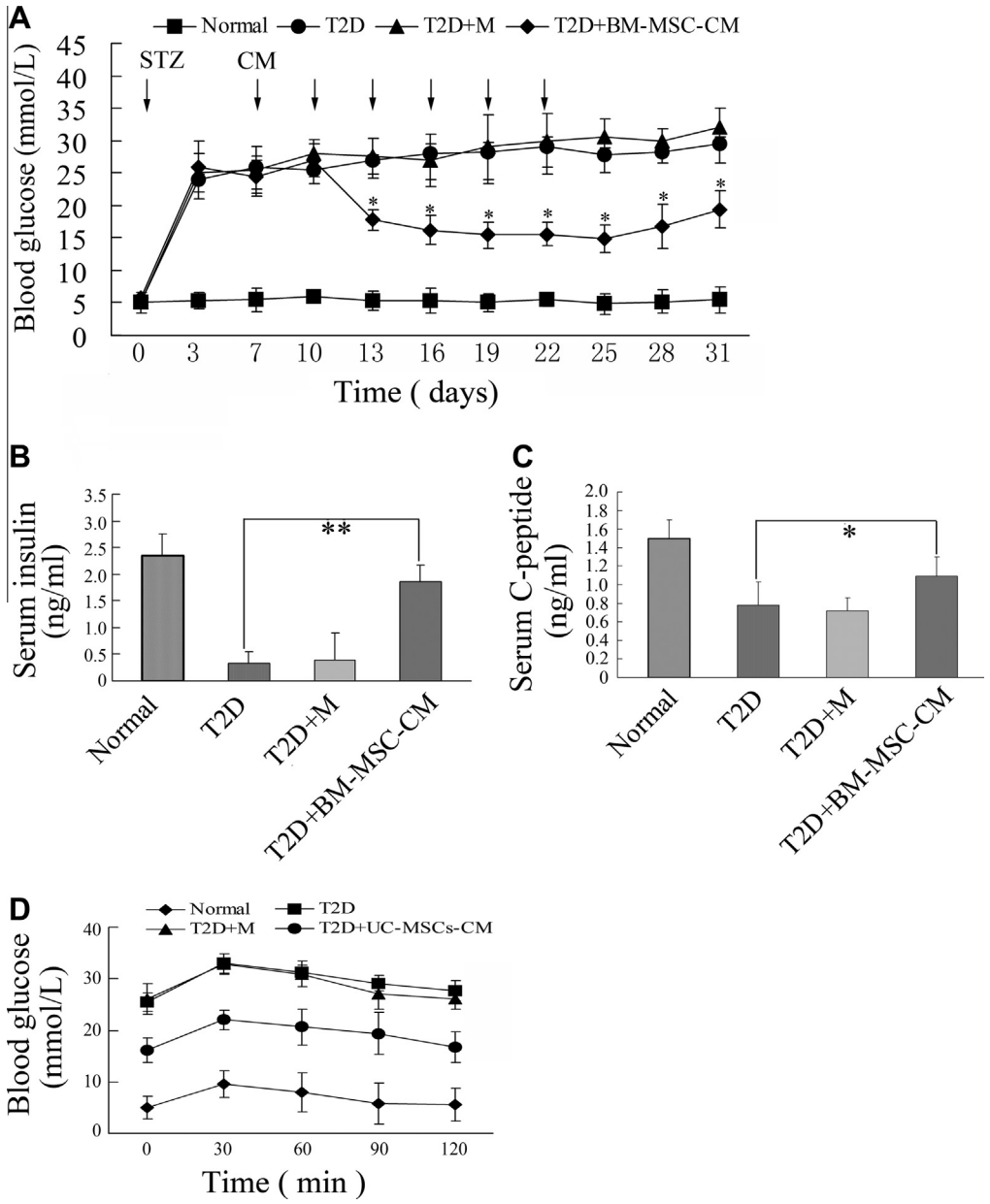

Fig. 4. Serial BM-MSC-CM infusions in T2D rats. (A) Blood glucose levels were determined consecutively in fasted rats. d, day. (B) Individual insulin levels in fasted and re-fed rats were evaluated by ELISA (n = 10 rats per group). (C) Individual C-peptide levels in fasted and re-fed rats were evaluated by ELISA (n = 10 rats per group). (D) Individual oral glucose tolerance was assessed by OGTTs and determining blood glucose levels 21 days after MSC-CM infusion. Data are shown as mean ± SD ($^{*}P < 0.05$ and $^{**}P < 0.01$).

were maintained at 15 ± 3.4 mmol/L from the second to the fourth CM infusion, and then increased gradually at 28 days (Fig. 4A). Meanwhile, the concentrations of serum insulin and C-peptide increased dramatically (Fig. 4B and C) in the CM-infusion group. OGTT results revealed clear improvements in glucose metabolism following CM infusion (Fig. 4D). These data confirmed that hyperglycemia T2D rats could be ameliorated by BM-MSCs CM infusion. We accordingly proposed that multiple BM-MSC infusions reversed hyperglycemia and restored damaged pancreatic islets, largely as the result of BM-MSC secretion of cytokines and growth factors. These results are consistent with several other reports [18–20].

4. Discussion

MSCs have been shown to exert effects of anti-diabetic potentially, however, they were unable to achieve normoglycemic by one or two MSC infusion in diabetic animal models [9–14]. The question addressed by the present study was whether multiple MSC infusion further ameliorated hyperglycemia in T2D rats. We administered serial allogenous BM-MSC infusions to STZ/high-fat diet-induced T2D rats. The results showed that hyperglycemia of T2D rats reduced to normal levels after multiple BM-MSC infusions. Analysis of insulin, C-peptide, insulin/glucagon staining and OGTTs all indicated the restoration of islet structure and function after multiple MSC treatments.

The recent study showed that administration of MSCs in T1D animal models could alleviate the high blood glucose syndromes for a short period, with signs of islets regeneration [9–11], and even multiple intravenous transplantations of MSCs effectively restore long-term blood glucose homeostasis [18]. However, T2D is commonly linked to obesity, which can cause insulin resistance and pancreatic β-cell dysfunction, and is a chronic and progressive disease. Meanwhile, evidences come from experimental and

clinical showed that inflammation is now accepted as one factor in the pathology of T2D, and possibly the underlying cause of b-cell death [21–26]. Consequently, administration of MSCs can also ameliorate hyperglycemia in T2D animal models [27,28]. Our previous studies demonstrated that MSC infusion improved the insulin sensitivity of insulin target tissue by upregulated GLUT4 protein expression and stimulating its translocation to the cellular membrane [13]. Insulin resistance and impaired glucose metabolism are commonly associated with decreased translocation of GLUT4 protein to the cell membrane in T2D [29]. And then, in the present study, multiple BM-MSC infusions can ameliorate hyperglycemia in T2D rats. Blood glucose levels were restored to near normal for at least 9 weeks.

It remains unclear how multiple BM-MSC infusion restored normal blood glucose in T2D rats. Since cellular differentiation appears to play a minor role in the therapeutic of BM-MSCs in this system [13], as well as the number of transplanted MSCs that home to and functionally integrate into the damaged tissue is generally too low to support a regeneration of the structure [11]. It is logical to hypothesize that paracrine release of cytokines and growth factors by infused BM-MSCs is responsible for the observed effects. In this study, we have observed that the BM-MSCs conditioned medium was also ameliorate hyperglycemia and partial restoration of pancreatic islet function in T2D rats. However, it is not restored to normal blood glucose and pancreatic islets function by administration of serially BM-MSC CM infusions via the tail vein once every 3 days in T2D rats because of its short half life. In view of the secretory effect depends on the survival time of the transplanted MSCs in vivo, we adopted a continuous administration of BM-MSCs biweekly strategy. The results demonstrated that the blood glucose of T2D rats close to normal levels after the fourth time infusion. Meanwhile, serials BM-MSC infusion also remarkably promoted regeneration of impaired pancreatic islets in T2D rats.

In conclusion, we verified that multiple MSC infusions could reverse hyperglycemia and restore injured pancreatic islets in T2D rats induced by high-fat diet and STZ. When multiple-MSC treatment was initiated in late-phase (5 week) T2D rats, the results were slightly different. Moreover, we demonstrated that cytokines and growth factors by infused BM-MSCs are responsible for the observed effects. The result showed that multiple MSC infusions may represent a useful clinical strategy for treatment of T2D patients.

Acknowledgments

This research was supported in part by the National Basic Science and Development Program [2012CB518103 and 2012CB518105], the 863 Projects of Ministry of Science and Technology of China [2011AA020113 and 2013AA020105], National Natural Science Foundation of China (81121004 and 81230041).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.117.

References

[1] J.E. Shaw, R.A. Sicree, P.Z. Zimmet, Global estimates of the prevalence of diabetes for 2010 and 2030, Diabetes Res. Clin. Pract. 87 (2010) 4–14.
[2] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 31 (Suppl. 1) (2008) S55–S60.
[3] U. Lakshmipathy, C. Verfaillie, Stem cell plasticity, Blood Rev. 19 (2005) 29–38.
[4] R.J. Deans, A.B. Moseley, Mesenchymal stem cells: biology and potential clinical uses, Exp. Hematol. 28 (2000) 875–884.
[5] A. Giordano, U. Galderisi, I.R. Marino, From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells, J. Cell. Physiol. 211 (2007) 27–35.
[6] F.E. Ezquer, M.E. Ezquer, D.B. Parrau, D. Carpio, A.J. Yanez, P.A. Conget, Systemic administration of multipotent mesenchymal stromal cells reverts hyperglycemia and prevents nephropathy in type 1 diabetic mice, Biol. Blood Marrow Transplant. 14 (2008) 631–640.
[7] Y.L. Si, Y.L. Zhao, H.J. Hao, X.B. Fu, W.D. Han, MSCs: biological characteristics, clinical applications and their outstanding concerns, Ageing Res. Rev. 10 (2011) 93–103.
[8] H. Song, B.W. Song, M.J. Cha, I.G. Choi, K.C. Hwang, Modification of mesenchymal stem cells for cardiac regeneration, Expert Opin. Biol. Ther. 10 (2010) 309–319.
[9] F. Ezquer, M. Ezquer, D. Contador, M. Ricca, V. Simon, P. Conget, The antidiabetic effect of mesenchymal stem cells is unrelated to their transdifferentiation potential but to their capability to restore Th1/Th2 balance and to modify the pancreatic microenvironment, Stem Cells 30 (2012) 1664–1674.
[10] R.H. Lee, M.J. Seo, R.L. Reger, J.L. Spees, A.A. Pulin, S.D. Olson, D.J. Prockop, Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice, Proc. Natl. Acad. Sci. USA 103 (2006) 17438–17443.
[11] A.M. Madec, R. Mallone, G. Afonso, E. Abou Mrad, A. Mesnier, A. Eljaafari, C. Thivolet, Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells, Diabetologia 52 (2009) 1391–1399.
[12] K.S. Park, Y.S. Kim, J.H. Kim, B. Choi, S.H. Kim, A.H. Tan, M.S. Lee, M.K. Lee, C.H. Kwon, J.W. Joh, S.J. Kim, K.W. Kim, Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation, Transplantation 89 (2010) 509–517.
[13] Y. Si, Y. Zhao, H. Hao, J. Liu, Y. Guo, Y. Mu, J. Shen, Y. Cheng, X. Fu, W. Han, Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: identification of a novel role in improving insulin sensitivity, Diabetes 61 (2012) 1616–1625.
[14] V.S. Urban, J. Kiss, J. Kovacs, E. Gocza, V. Vas, E. Monostori, F. Uher, Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes, Stem Cells 26 (2008) 244–253.
[15] M.J. Reed, K. Meszaros, L.J. Entes, M.D. Claypool, J.G. Pinkett, T.M. Gadbois, G.M. Reaven, A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat, Metabolism 49 (2000) 1390–1394.
[16] A.S. Mageed, D.W. Pietryga, D.H. DeHeer, R.A. West, Isolation of large numbers of mesenchymal stem cells from the washings of bone marrow collection bags: characterization of fresh mesenchymal stem cells, Transplantation 83 (2007) 1019–1026.
[17] K. Bieback, S. Kern, H. Kluter, H. Eichler, Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood, Stem Cells 22 (2004) 625–634.
[18] J.H. Ho, T.C. Tseng, W.H. Ma, W.K. Ong, Y.F. Chen, M.H. Chen, M.W. Lin, C.Y. Hong, O.K. Lee, Multiple intravenous transplantations of mesenchymal stem cells effectively restore long-term blood glucose homeostasis by hepatic engraftment and beta-cell differentiation in streptozocin-induced diabetic mice, Cell Transplant. 21 (2012) 997–1009.
[19] S. Sadat, S. Gehmert, Y.H. Song, Y.S. Yen, X.W. Bai, S. Gaiser, H. Klein, E. Alt, The cardioprotective effect of mesenchymal stem cells is mediated by IGF-1 and VEGF, Biochem. Biophys. Res. Commun. 363 (2007) 674–679.
[20] Y.X. Xu, L. Chen, R. Wang, W.K. Hou, P. Lin, L. Sun, Y. Sun, O.Y. Dong, Mesenchymal stem cell therapy for diabetes through paracrine mechanisms, Med. Hypotheses 71 (2008) 390–393.
[21] B. Brooks-Worrell, J.P. Palmer, Is diabetes mellitus a continuous spectrum?, Clin Chem. 57 (2011) 158–161.
[22] B.M. Brooks-Worrell, J.L. Reichow, A. Goel, H. Ismail, J.P. Palmer, Identification of autoantibody-negative autoimmune type 2 diabetic patients, Diabetes Care 34 (2011) 168–173.
[23] A.B. Goldfine, V. Fonseca, S.E. Shoelson, Therapeutic approaches to target inflammation in type 2 diabetes, Clin. Chem. 57 (2011) 162–167.
[24] D. Mathis, S.E. Shoelson, Immunometabolism: an emerging frontier, Nat. Rev. Immunol. 11 (2011) 81.
[25] R.G. Naik, J.P. Palmer, Latent autoimmune diabetes in adults (LADA), Rev. Endocr. Metab. Disord. 4 (2003) 233–241.
[26] S.E. Shoelson, J. Lee, A.B. Goldfine, Inflammation and insulin resistance, J. Clin. Invest. 116 (2006) 1793–1801.
[27] R. Jiang, Z. Han, G. Zhuo, X. Qu, X. Li, X. Wang, Y. Shao, S. Yang, Z.C. Han, Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study, Front. Med. 5 (2011) 94–100.
[28] A. Pileggi, Mesenchymal stem cells for the treatment of diabetes, Diabetes 61 (2012) 1355–1356.
[29] A. Zisman, O.D. Peroni, E.D. Abel, M.D. Michael, F. Mauvais-Jarvis, B.B. Lowell, J.F. Wojtaszewski, M.F. Hirshman, A. Virkamaki, L.J. Goodyear, C.R. Kahn, B.B. Kahn, Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance, Nat. Med. 6 (2000) 924–928.