Effects of autologous adipose-derived stem cell infusion on type 2 diabetic rats

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Abstract. The effects and possible mechanisms of adipose-derived stem cells (ASC) infusion on type 2 diabetic rats were investigated in this study. Twenty normal male Sprague-Dawley rats were included in normal control group, and 40 male diabetic rats were randomly divided into diabetic control group and ASC group (which received ASC infusion). After therapy, levels of fasting plasma glucose (FPG), HbA1c, serum insulin and C-peptide, recovery of islet cells, inflammatory cytokines, and insulin sensitivity were analyzed. After ASC infusion, compared with diabetic control group, hyperglycemia in ASC group was ameliorated in 2 weeks and maintained for about 6 weeks, and plasma concentrations of insulin and C-peptide were significantly improved \((P<0.01)\). Number of islet β cells and concentration of vWF in islets in ASC group increased, while activity of caspase-3 in islets was reduced. Moreover, concentrations of TNF-α, IL-6 and IL-1β in ASC group obviously decreased \((P<0.05)\). The expression of GLUT4, INSR, and phosphorylation of insulin signaling molecules in insulin target tissues were effectively improved. ASC infusion could aid in T2DM through recovery of islet β cells and improvement of insulin sensitivity. Autologous ASC infusion might be an effective method for T2DM.

Key words: Adipose-derived stem cells, Type 2 diabetes mellitus, Islet β cells, Insulin resistance

TYPE 2 DIABETES MELLITUS (T2DM) accounts for more than 90% of all diabetic patients and is characterized by insulin resistance, hyperglycemia, systemic low-grade inflammation, and relative lack of insulin. Most T2DM patients experience β-cell exhaustion several years after diagnosis, and thus have to accept insulin therapy. However, insulin therapy is inconvenient for patients and does not completely prevent the development of diabetic complications. Stem cells are an attractive option to ameliorate diabetes for its abundant source and potential to acquire glucose-dependent insulin secretory function. Adult stem cells are an especially good candidate for its safety in terms of tumorigenicity and ethical concerns.

Adipose-derived stem cells (ASC) have been studied over the past decade as an alternative adult stem cell source for autologous cell replacement. ASC can be isolated from small amounts of adipose tissue, efficiently expanded to achieve a very large number after 3 to 4 passages, and have the advantage of low immunogenicity and high multipotency. Their immunmodulatory function, homing and migratory patterns, as well as previous clinical trials, have suggested that these cells are efficient and safe for treating several classes of autoimmune diseases [1-4].

The effect of stem cell therapy on type 2 diabetes has been suggested by several studies. Mesenchymal stem cells (MSC) from bone marrow were shown to improve blood glucose levels and survival of type 2 diabetic rats [5]. Pancreatic β-like cells derived from induced-pluripotent stem cells also exhibited a reversal of hyperglycemia in mouse type 2 diabetic models [6]. Clinical trials also demonstrated the effect and safety of stem cell implantation using autologous bone marrow cells or placenta-derived mesenchymal stem cells in patients with type 2 diabetes [7-9]. However, most...
T2DM animal models and patients treated with stem cells were in the early phase of diabetes and although the therapeutic effect is exciting, the exact mechanisms are still unclear.

Obesity and insulin resistance are important elements in the pathogenesis of type 2 diabetes. In populations with a high prevalence of T2DM, insulin resistance is well established long before the development of any impairment in glucose homeostasis, particularly in subjects with abdominal or ectopic (liver, muscle) fat accumulation. As for obese patients with longer history of diabetes (e.g., five years or longer), they have to be faced with deteriorating β-cell function and continued insulin resistance [10]. So, in the present study, we induced T2DM in a rat model by a high-fat diet combined with streptozotocin (STZ) administration [11], and performed MSC infusion at 28 days after STZ injection, in order to investigate the therapeutic effect and possible mechanisms of ASC infusion on type 2 diabetes of longer history.

Materials and Methods

Animals

Eighty male Sprague-Dawley rats (8 weeks old, weighing 180–200 g) were obtained from SLAC (Shanghai Laboratory Animal Center), Shanghai. Animals were maintained in metabolic cages, fed on rat chow and maintained under optimal ambience of temperature (22–23°C), light (12 h dark/12 h light cycle), oxygen, humidity (60%), and ventilation until sacrificed. All experimental procedures and protocols for animals conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethical Committee, Qingdao and the Ethics Committee of the Affiliated Hospital of Qingdao University.

Upon arrival, all rats were allowed free access to food and water for one week to allow their adaptation to the environment. A high-fat diet and STZ intraperitoneal injection were then administered to 60 rats to induce type 2 diabetes [11]. The remaining 20 rats fed with normal diet served as normal controls. A high-fat diet consisting of 58% fat, 17% carbohydrates, and 25% protein, with a total caloric value 54.3 kJ/kg and a normal pellet diet consisting of 5% fat, 53% carbohydrates, 23% protein, with total caloric value 25 kJ/kg, were ordered from the Stoyer Center of SLAC. After consuming the high-fat diet for 4 weeks, rats in diabete mellitus (DM) group were intraperitoneally injected with 35 mg/kg of STZ. Ten days after STZ injection, fasting blood glucose (FPG) of rats in DM group were measured after an 8-hour fast, insulin release tests and islet histologic examination were performed to confirm the T2DM rat model. The diabetic rats were allowed to continue to feed on a high-fat diet until the end of the study.

Experimental design

Forty rats fed with a high-fat diet and STZ intraperitoneal injection were confirmed as diabetic and randomly divided into two groups (n=20), including ASC group and diabetic control group. Rats in ASC group were treated with an intravenous infusion of ASC through vena caudalis at the 28th day after STZ injection, the cell number was 2×10^6/rat and cells were suspended in 0.5 mL physiological saline. Rats in diabetic control group were as diabetic controls and infused with 0.5 mL physiological saline through vena caudalis.

Isolation and culture of ASC

Fresh adipose tissues (5 mL) were obtained from the inguinal fat pads of each diabetic rat and were washed with phosphate buffered saline (PBS). Adipose tissues were then digested with 0.1% type II collagenase for 1 h at 37ºC on a shaker (200 rpm). After 2 washes by centrifugation at 1500 rpm for 5 min in PBS, cells were plated in 75-cm² tissue-culture flasks in low glucose DMEM (LG-DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum. Cells were kept in a humidified 5% CO₂ incubator at 37ºC, medium was refreshed every 3-4 days. Adherent cells were split with 0.25% trypsin+0.02% EDTA (Sigma-Aldrich, St. Louis, MO, USA) when they were 75% confluent. ASC at passage 3 were used in the experiment.

Characterization of ASC

ASC were characterized through their phenotype and their potential to differentiate into osteoblasts and adipocytes. For flow cytometry analysis, ASC at passage 3 were harvested and respectively incubated with fluorescein isothiocyanate (FITC)-conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies: CD90, CD44, CD105, CD73, CD34 and CD45. Flow cytometry analysis was performed on a FACSCalibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA).

To investigate their osteogenic and adipogenic potency, ASC were seeded in 6-well plates at 1×10^5
cells/mL per well. When they reached 80% confluence, the cells were induced using osteogenic medium, containing 10 mmol/L beta-sodium glycerophosphate, 50 μg/mL ascorbic acid and 10 mmol/L dexamethasone in standard medium (containing DMEM/F12, 10% fetal bovine serum, 100 mg/mL streptomycin and 100 U/mL penicillin) and adipogenic medium, including 1 μmol/L hexadecadrol, 10 μmol/L insulin, 200 μmol/L indomethacin, and 0.5 mmol/L isobutyl-methylxanthine for 3 weeks. Alizarin Red staining and Oil Red O staining were used to identify the osteogenic and adipogenic potential, respectively, after 3 weeks.

Quantification of islet cells

Following immunofluorescence staining of the islets, the numbers of different cell types (α, β cells) and total islet cells in each islet were manually counted from the islet microscopy images. Numbers of α and β cells in the islets were estimated using sections sampled randomly and systematically from the whole serial sections. In order to verify the exact number of islet cells, we repeated this quantitative analysis with ImageJ software. For each cell type, approximately 50 islets per rat were counted in each group.

Caspase-3 activity assay

Caspase-3 activity in lysates from pancreatic islets was determined using the Caspase-3 Colorimetric Assay Kit (Nanjing KeyGen Biotech. Co., Ltd., Nanjing, China), according to the manufacturer’s protocol. Caspase-3 activity was expressed in terms of absorbance units (OD 405 nm) per milligram of protein.

Examination of cytokines

Levels of proinflammatory cytokines TNF-α, IL-1β, IL-6 and von Willebrand factor (vWF) in the pancreatic tissue homogenates were determined by a quantitative sandwich enzyme technique using Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA). The minimum measurable limit of each cytokine was 16 pg/mL for TNF-α, 42.5 pg/mL for IL-1β, 67 pg/mL for IL-6, and 0.1 ng/mL for vWF. Samples were assayed in triplicates and the mean absorbance was calculated from the standard curve.

Detection of INSR, GLUT4, IRS-1, PDK1 and PKCζ by Western blot analysis

Total proteins were extracted from samples of skeletal muscle, adipose tissue and liver by PRO-PREP Protein Extraction Kit (iNtRON Biotechnology, Kyungki-Do, Korea). Aliquots containing 50 mg of protein were separated by 8% SDS-PAGE, and after electrophoresis, transferred to PVDF membrane (Millipore Corp., Billerica, MA, USA). Thereafter, the membranes were washed with TBS solution and blocked for 30 min in 5% free fat milk dissolved in 1× TBST (TRIS-base, NaCl and 0.001% Tween-20 with pH 7.6). After blocking, the membranes were incubated overnight at 4°C with the corresponding primary antibody diluted in the same blocking solution. Western blot analyses were performed using the monoclonal antibodies against insulin receptor (INSR), glucose transporter-4
(GLUT4), total or phosphorylated insulin receptor substrate 1 (IRS-1, Tyr612), 3’-phosphoinositide-dependent kinase 1 (PDK1, Ser241) and protein kinase C ζ (PKCζ, Thr410), respectively. Anti-rat β-actin was used as control. POD-conjugated secondary antibody and BM chemiluminescence western blotting substrate (Roche Diagnostics, Mannheim, Germany) were used to visualize bands on an X-ray sensitive film (Roche Diagnostics, Mannheim, Germany). Western blots were repeated at least three times and a representative blot from consistent triplicates was chosen for the figures. Graphs were prepared to represent the intensity of the bands on the x-ray films which were quantified using the Image J software (Fujifilm Science Lab, Madrid, Spain) and the average of three independent replicates was calculated.

**Statistical analysis**

All statistical analyses were performed using SPSS version 15.0 software (SPSS Inc., Chicago, USA). Results were expressed as mean±standard deviation (SD) unless otherwise designated. Differences between groups were tested by one-way ANOVA and post hoc analysis with Bonferroni correction for multiple comparisons. For parameters where repeated measurements were taken over time (e.g. FPG), a two-way repeated-measure ANOVA was performed. A P<0.05 was considered to be statistically significant.

**Results**

**Characterization of ASC and animals**

ASC isolated from inguinal adipose tissue of diabetic rats were adhered to the plastic surface of culture flasks and had a spindle-shaped morphology. The cells after passage three were of high purity, and expressed CD44+ (75.34%), CD105+ (79.26%), CD90+ (85.77%), CD73+ (89.63%), CD34− (0.34%) and CD45− (0.14%). Immunophenotyping and differentiation of ASC are shown in Fig. 1. Success of fat-fed, STZ-induced T2DM rat model was confirmed by fat body, hyperglycemia (FPG ≥ 11.1 mmol/L, twice), and morphological destruction of pancreatic islets. Messy fur, lags in response, polydipsia, polyphagia, urorrhagia, nastiness, and hypoergy were observed as expected among rats in diabetic groups. There were no significant differences

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**Fig. 1** ASC and differentiations

Upper: ASC after the third passage were of high purity, and expressed CD44+, CD105+, CD90+, CD73+, CD34− and CD45−.

Lower: ASC at passage 3, adipogenic differentiation (200×, stained with Oil Red O) and osteogenic differentiation (200×, stained with Alizarin Red S).
Infusion of ASC relieved the hyperglycemia of diabetic rats

During the whole study, rats in normal group were good as before, as their hydroposia, cibation, and body weight increased gradually, while rats in diabetic control group showed persistent hyperglycemia, gradual decrease in body weight and higher mortality. As for rats in ASC group, after ASC infusion, their body weight increased gradually. Moreover, their FPG began to decrease, reached the lowest level in two weeks, and was at that level sustained for six weeks. Also, the level of HbA1c of rats in ASC group was statistically lower compared to diabetic control group \( (P<0.05) \) but higher than normal control group \( (P<0.05) \), as shown in Fig. 2.

Concentrations of serum insulin and C-peptide were examined at different time points throughout the study. After STZ injection, concentrations of serum insulin and C-peptide of rats in ASC group and diabetic control group were lower than normal control group \( (P<0.05) \), while after ASC infusion, concentrations of serum insulin and C-peptide of rats in ASC group were slightly higher compared to diabetic control group \( (P<0.05) \), as shown in Fig. 2.

Infusion of ASC repaired islet cells by reducing the apoptosis of islet cells and promoting islet revascularization

Histopathologic analysis was done to assess the influence of ASC infusion on the late phase of STZ-induced islet damage. A small increase of islet \( \beta \) cells in ASC group was observed as expected when compared with diabetic control group. As shown in Fig. 3, the average number of \( \beta \) cells in ASC group was more than that in diabetic control group \( (P=0.032) \), but lower than normal control group \( (P=0.01) \). The average number of \( \alpha \) cells in ASC group increased after therapy and was higher compared to normal control group \( (P=0.019) \).

Caspase-3 activity in pancreatic islets was analyzed to explain the possible mechanisms of ASC infusion on islet cells. Eight weeks after therapy, the caspase-3 activity of pancreatic islet in diabetic control group was significantly higher than normal control group \( (P=0.03) \), as shown in Fig. 3), while activity of caspase-3 in ASC group was much lower compared to diabetic control group \( (P=0.038) \), but still higher compared to normal control group \( (P=0.031) \). The results suggested that ASC infusion might repair islet cells by reducing islet cell apoptosis in diabetic rats.

To assess the effect of ASC infusion on islet revascularization in diabetic rats, we examined the concentrations of vWF in pancreatic islets. The concentrations of vWF of pancreatic tissue in ASC group were higher than normal control group and diabetic control group after therapy \( (P=0.034 \) and \( P=0.016 \), as shown in Fig. 3). This indicated that ASC infusion might promote the revascularization of pancreatic islets to repair islet cells.

Infusion of ASC modulated the inflammation in pancreatic tissue

To assess the influence of ASC infusion on STZ-induced tissue inflammation, we examined the concentrations of proinflammatory cytokines TNF-α, IL-1β and IL-6 in pancreatic tissue lysates. Concentrations of TNF-α, IL-1β, and IL-6 in diabetic control group were higher compared to normal control group \( (P=0.013, 0.022, \) and \( 0.03) \), respectively. After ASC infusion, concentrations of TNF-α, IL-1β and IL-6 in ASC group were lower compared to diabetic control group \( (P=0.032, 0.027, \) and \( 0.028) \), but higher compared to normal control group \( (P=0.04, 0.038, \) and \( 0.031 \), as shown in Fig. 4). The results suggested that ASC infusion could modulate the inflammation in pancreatic tissue.

ASC infusion improved insulin sensitivity in diabetic rats

We performed hyperinsulinemic-euglycemic clamp studies under euglycemic conditions to evaluate the effect of ASC infusion on insulin sensitivity in the late phase of fat-fed and STZ-induced T2DM rats. Comparisons of basal/clamp HGP, GDR, and GIR in each group were shown in Fig. 5. GDR and GIR were significantly increased in rats in ASC group. There was no significant difference in basal HGP between rats in ASC group and diabetic control group, but clamp HGP was significantly lower in rats in ASC group. Moreover, the levels of the IR index and HBCI in rats in ASC group indicated their reduced insulin resistance and improved \( \beta \)-cell function. All these results suggested that MSC infusion could improve insulin sensitivity in the late phase of diabetic rats.

Infusion of ASC influenced the expression of GLUT4 and INSR in insulin target tissues

We examined the expressions of GLUT4 and
Fig. 2  ASC infusion relieved the hyperglycemia of diabetic rats

(A) After therapy, compared with diabetic control group, body weight of rats in ASC group increased gradually (*P<0.05). Two weeks after ASC infusion, FPG in ASC group reached the lowest level and sustained for 6 weeks (*P<0.05, **P<0.01).

(B) Levels of HbA1c of rats in ASC group were lower compared to diabetic control group (**P<0.01) but higher than normal control group (*P<0.05).  

(C) Concentrations of serum insulin and C-peptide of rats in ASC group were significantly lower compared to normal control group (*P=0.021 and **P=0.029) at 4 weeks of the study.  

(D) After ASC infusion, concentrations of serum insulin and C-peptide of rats in ASC group were higher compared to diabetic control group, but still lower than normal control group (*P<0.05).
Fig. 3  ASC infusion repaired islet cells by reducing the apoptosis of islet cells and promoting islet revascularization

(A) Eight weeks after therapy, the average number of β cells in ASC group was more than that in diabetic control group (*P=0.032), but still lower than normal control group (*P=0.01). The average number of α cells in diabetic control group and ASC group was higher than that in normal control group (*P=0.027 and 0.019).  
(B) Caspase-3 activity of pancreatic tissue in diabetic control group was higher than normal control group (*P=0.03). Activity of caspase-3 in ASC group was lower compared to diabetic control group eight weeks after therapy (*P=0.038), but still higher than normal control group (*P=0.031).  
(C) Concentration of vWF in ASC group was higher compared to diabetic control group and normal control group after therapy (*P=0.034 and P = 0.016).
of stem cells with an inherent ability for self-renewal and differentiation potential. Except for similarity to MSC from umbilical cord and bone marrow, ASC have great advantages for cell preparation because of the easier and safer access to adipose tissue and their higher proliferative activity with greater production of vascular endothelial cell growth factor (VEGF) and hepatocyte growth factor (HGF) than bone marrow derived MSC (BM-MSC) [14]. Tissue source has a greater influence on gene expression profiles than the different culture methods. Gene array and differentiation analysis revealed that 1% of genes were differentially expressed between ASC and BM-MSC [15]. Adipose tissue contains a diversity of cell types, including adipocytes, stromal cells, vascular endothelial cells (ECs), pericytes, and resident blood-derived cells. The higher level of heterogeneity of ASC suggests that those cells do represent MSC that have more lineage capabilities than BM-MSC [16, 17]. However, the effects of diabetes on ASC leads to a gradual depletion of the ASC pool, as with BM-MSC [18, 19].

More studies have confirmed that MSC therapy could effectively ameliorate both type 1 and type 2 diabetes [20-22], while there are few reports on the effects of ASC on the late phase of type 2 diabetes. In the present study, we induced T2DM in a rat model by a high-fat diet combined with STZ administration and performed ASC infusion at the 28th day after STZ injection. We found that ASC infusion could effectively ameliorate hyperglycemia in T2DM rats, which was consistent with other reports [1, 2, 23]. Furthermore, our results of hyperinsulinemic-euglycemic clamp studies demonstrated that ASC infusion modulated the insulin sensitivity in skeletal muscle and adipose and liver tissues after therapy to investigate the possible mechanisms involved in the effect of ASC infusion on insulin sensitivity. Total membrane fractions of GLUT4 and INSR in skeletal muscle and liver and adipose tissues were dramatically decreased in diabetic control group compared to normal control group ($P<0.01$). However, after ASC infusion, both fractions were increased in ASC group, as shown in Fig. 6. The results suggested that ASC might improve the insulin sensitivity of target tissues through mediating the membrane expression of GLUT4 and INSR.

**Discussion**

It is widely recognized that MSC include a number of stem cells with an inherent ability for self-renewal and differentiation potential. Except for similarity to MSC from umbilical cord and bone marrow, ASC have great advantages for cell preparation because of the easier and safer access to adipose tissue and their higher proliferative activity with greater production of vascular endothelial cell growth factor (VEGF) and hepatocyte growth factor (HGF) than bone marrow derived MSC (BM-MSC) [14]. Tissue source has a greater influence on gene expression profiles than the different culture methods. Gene array and differentiation analysis revealed that 1% of genes were differentially expressed between ASC and BM-MSC [15]. Adipose tissue contains a diversity of cell types, including adipocytes, stromal cells, vascular endothelial cells (ECs), pericytes, and resident blood-derived cells. The higher level of heterogeneity of ASC suggests that those cells do represent MSC that have more lineage capabilities than BM-MSC [16, 17]. However, the effects of diabetes on ASC leads to a gradual depletion of the ASC pool, as with BM-MSC [18, 19].

![Figure 4](image-url)  
**Fig. 4** ASC infusion modulated the inflammation in pancreatic tissue

Concentrations of TNF-α, IL-1β and IL-6 in diabetic control group were higher compared to normal control group ($**P=0.013, 0.022$ and 0.03). After ASC infusion, concentrations of TNF-α, IL-1β and IL-6 in ASC group were lower than diabetic control group ($*P=0.032, 0.027$ and 0.028), but still a little higher than normal control group ($*P=0.04, 0.038$ and 0.031).
Fig. 5  ASC infusion improved insulin sensitivity in diabetic rats

Insulin sensitivity of each group was measured by hyperinsulinemic-euglycemic clamp.  (A, B) GDR, GIR and HGP of each group at insulin infusion rates of 8 mU/kg/min.  (C) HBCI of each group, HOMA-β (HBCI) = (20 × FINS [in units/L])/(FBG [in mmol/L] - 3.5).  (D) IR index of each group, HOMA-IR index = (FBG [in mmol/L] × FINS [in units/L])/22.5.  Values are means±SD.  *P < 0.05 and **P < 0.01.
Recent studies have demonstrated the homing properties of i.v. administered ASC to cell-damaged areas in diabetic nephropathy rats, cardiomyocytes, and carotid artery injury animal model [28-30]. Although most cells remained in spleen and thymus, a relatively high percentage of ASC were found in injured region. Moreover, Yang et al. had found that engrafted ASC homed to retina and differentiated into photoreceptor-like cells and astrocyte-like cells induced by diabetic retinopathy, and ameliorated the integrity of the blood-retinal barrier [31]. Based on the above findings, we considered that ASC could home to the damaged islets to play their roles. Further experiments need to be done on labeled-ASC homing, transdifferentiation to β cells or endothelial cells in vivo, and restoring the function of tissue resident stem cells.

Autopsy studies of patients with T2DM have revealed that the real determinant of lower β-cell mass in T2DM is an increased rate of apoptosis [32]. We considered 28 days after STZ injection as the late phase of diabetes according to a previous study [23]. A single infusion of ASC ameliorated the hyperglycemia of diabetic rats and maintained that level for about six weeks, while the concentrations of serum insulin and C-peptide in ASC group increased compared to diabetic control group and maintained that level until the end of the study. This might due to the partial restoration of islet function, and the increase of islet β cells after ASC infusion in our study also confirmed it. Nevertheless, the precise mechanisms underlying the effect are still unclear. We think it was not only a result of reduced glucose toxicity, for it was not very effective at retarding the progressive β-cell dysfunction, inhibiting inflammation and cell apoptosis, and promoting angiogenesis. Accumulating evidence indicated that the paracrine signaling initiated by MSC, which involved secretion of various angiogenic growth factors and cytokines, anti-inflammatory and anti-apoptotic molecules, was responsible for MSC therapy [24-27].

Recent studies have demonstrated the homing properties of i.v. administered ASC to cell-damaged areas in diabetic nephropathy rats, cardiomyocytes, and carotid artery injury animal model [28-30]. Although most cells remained in spleen and thymus, a relatively high percentage of ASC were found in injured region. Moreover, Yang et al. had found that engrafted ASC homed to retina and differentiated into photoreceptor-like cells and astrocyte-like cells induced by diabetic retinopathy, and ameliorated the integrity of the blood-retinal barrier [31]. Based on the above findings, we considered that ASC could homed to the damaged islets to play their roles. Further experiments need to be done on labeled-ASC homing, transdifferentiation to β cells or endothelial cells in vivo, and restoring the function of tissue resident stem cells.

Fig. 6 ASC infusion influenced expression of GLUT4 and INSR in insulin target tissues

Total membrane fractions of GLUT4 and INSR in skeletal muscle and liver and adipose tissues were decreased in diabetic control group compared to normal control group ("P<0.01). After ASC infusion, both fractions in ASC group were higher than diabetic control group but lower than normal control group (*P<0.05).
ASC and T2DM

This was consistent with the findings that ASC could transdifferentiate toward endothelial cells in vitro and release angiogenic factors such as VEGF, HGF, and vWF in vivo [33, 34]. We thus concluded that ASC could restore islet function by reducing the apoptosis of islet cells and promoting islet vascularization.

After ASC infusion, concentrations of TNF-α, IL-6 and IL-1β of T2DM rats significantly decreased. Adipose tissue inflammation is a major contributor to the pathogenesis of obesity-associated insulin resistance [10]. Excess fat stored in adipose tissue results in adipose tissue dysfunction which is characterized by marked changes in secretion of inflammatory cytokines [35, 36]. Pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1β, act in an autocrine and paracrine manner to promote insulin resistance via a number of mechanisms, including activation of inflammatory pathways, serine phosphorylation of the insulin receptor and insulin receptor substrate 1, and increased serum and hepatic triacylglycerol levels [37-39]. Our results demonstrated that ASC might improve insulin resistance by modulating inflammation and inflammatory cytokines.

We also found that the total amounts of GLUT4 and INSR on the cell membrane in skeletal muscle and liver and adipose tissues were restored after ASC infusion, the same as the increased phosphorylation of IRS-1, PDK1, and PKCζ in insulin signaling transduction. To date the molecular mechanisms of insulin resistance in diabetes have been explored extensively. Elevation of GLUT4 protein on cell membrane of peripheral insulin target tissues are responsible for improvement in sen-
sitivity to insulin action. IRS-1, PDK1, and PKCζ are also important for conferring insulin signaling transduction and glucose uptake besides protein kinase Akt [40]. This result was consistent with Si’s study, which found MSC infusion could restore the concentration of total GLUT4 and promote GLUT4 translocation to the cell membrane [23].

In conclusion, we confirmed the therapeutic roles and possible mechanisms involved in the effect of ASC in treating late phase T2DM rats. ASC infusion could effectively ameliorate hyperglycemia, restore islet β cells by reducing cell apoptosis and promoting islet angiogenesis, and improve insulin resistance of T2DM. These findings provide an important basis for exploring ASC infusion in T2DM therapy. However, this study is a preliminary experiment of our research project on the effect and mechanisms of ASC on T2DM. Further experiments on labeled-ASC homing, transdifferentiation to β cells or endothelial cells in vivo, restoring function of tissue resident stem cells, and the exact mechanisms of improving insulin resistance are ongoing.

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We wish to express our appreciation for all the work done by Lin Han in this study.

Ethical Standards

All experimental procedures and protocols for animals conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethical Committee, Qingdao and the Ethics Committee of the Affiliated Hospital of Qingdao University.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


