Infusion of Mesenchymal Stem Cells Ameliorates Hyperglycemia in Type 2 Diabetic Rats

Identification of a Novel Role in Improving Insulin Sensitivity

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Infusion of mesenchymal stem cells (MSCs) has been shown to effectively lower blood glucose in diabetic individuals, but the mechanism involved could not be adequately explained by their potential role in promoting islet regeneration. We therefore hypothesized that infused MSCs might also contribute to amelioration of the insulin resistance of peripheral insulin target tissues. To test the hypothesis, we induced a diabetic rat model by high-fat diet/streptozotocin (STZ) administration, performed MSC infusion during the early phase (7 days) or late phase (21 days) after STZ injection, and then evaluated the therapeutic effects of MSC infusion and explored the possible mechanisms involved. MSC infusion ameliorated hyperglycemia in rats with type 2 diabetes (T2D). Infusion of MSCs during the early phase not only promoted β-cell function but also ameliorated insulin resistance, whereas infusion in the late phase merely ameliorated insulin resistance. Infusion of MSCs resulted in an increase of GLUT4 expression and an elevation of phosphorylated insulin receptor substrate 1 (IRS-1) and Akt (protein kinase B) in insulin target tissues. This is the first report of MSC treatment improving insulin sensitivity in T2D. These data indicate that multiple roles and mechanisms are involved in the efficacy of MSCs in ameliorating hyperglycemia in T2D. ***Diabetes*** **61:1616–1625, 2012**

Diabetes has become one of the most serious threats to global public health, with an estimated worldwide prevalence of 171 million cases among the adult population (1). Type 2 diabetes (T2D) is the most common form of diabetes and accounts for ~90–95% of all existing diabetic cases (1,2). The main etiologies of T2D include insulin resistance in target tissues, relatively insufficient secretion of insulin, and subsequent decline of pancreatic β-cell function (3). Current traditional therapies for T2D include insulin sensitizers and exogenous supply of insulin (4). Although these drugs can ameliorate hyperglycemia or temporarily improve the response to insulin in target tissues, they are not very effective at retarding the progressive β-cell dysfunction. Strategies to ameliorate peripheral insulin resistance and simultaneously promote β-cell regeneration would provide future treatment options for patients with T2D. Recent advances in identification of stem cells that possess the potential to differentiate into insulin-producing cells and improve pancreatic regeneration generated hope that this therapeutic notion could become a reality (5,6).

Mesenchymal stem cells (MSCs) are one of the most important multipotent adult stem cells. Owing to their capacities to differentiate into replacement cells in damaged tissues, modulate their local environment, activate endogenous progenitor cells, and secrete various factors (7,8), MSCs appear to hold great promise for treatment of disease and regeneration of injured tissues, such as in ischemic diseases, neurologic disorders, and diabetes, among others (5,9–11). As summarized in our review (7), a total number of seven registered clinical trials on type 1 and/or type 2 diabetes in phase I/II can be found on the website for clinicaltrials.gov (http://www.clinicaltrials.gov). In these clinical trials, MSCs exhibited exciting therapeutic effects in diabetic volunteers (12). Moreover, studies in diabetic models have also shown that MSCs are able to lower blood glucose levels (5,13,14). Nevertheless, the precise mechanisms underlying these effects are still poorly understood. Because few MSCs could be found to differentiate into functionally competent β-cells in vivo (~1.7–3% of infused MSCs) (15), it seems likely that there might be another mechanism underlying the therapeutic effect of MSCs in diabetes. Recent studies have shown that MSCs can produce a variety of trophic cytokines to improve the microenvironment of the pancreas and promote expansion of endogenous pancreatic stem cells (14,16). However, these findings were still not adequate to explain the therapeutic contribution of MSCs to T2D, which is prominently characterized by peripheral insulin resistance. Whether infused MSCs could improve the insulin sensitivity of peripheral insulin-target tissues (the principal ones being muscle, adipose, and liver tissues) is unknown. To date, no relevant reports have been published. Based on current knowledge, molecules such as insulin receptor substrate 1 (IRS-1), protein kinase Akt (protein kinase B), and GLUT4 are crucial for conferring insulin signaling transduction and glucose uptake. Reduced expression of GLUT4 and dysregulation of IRS-1 and Akt phosphorylation underscore the mechanism involved in insulin resistance (17,18). Up until now, the relationship of MSCs to insulin resistance and their influence on insulin signaling has been unknown.

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To investigate the possible therapeutic mechanisms involved in MSC infusion, we induced T2D in a rat model by a high-fat diet (HFD) combined with streptozotocin (STZ) administration, performed MSC infusion at different times after STZ injection, and then measured the effect of MSC infusion on hyperglycemia using hyperinsulinemic-euglycemic clamp studies that we developed. Our results showed that infused MSCs reduced blood glucose levels in diabetic rats through multiple mechanisms, including promoting β-cell function, improving insulin sensitivity possibly by upregulating GLUT4 expression, and elevating phosphorylated IRS-1 and Akt levels in insulin target tissues.

RESEARCH DESIGN AND METHODS

Induction of rat T2D model. The fat-fed, STZ-induced rat T2D model was established as previously described (19).

Isolation, culture, and identification of bone marrow–derived MSCs. Bone marrow–derived MSCs (BM-MSCs) were isolated, purified, and identified as described previously (20,21).

BM-MSC administration. MSCs between passage three and six were used for infusion. To investigate the relationship between infusion phase and the effectiveness of MSCs, we performed MSC infusion (2×10^6 MSCs suspended in 0.2 mL physiological saline) in T2D rats through the tail vein at 7 (early phase) or 21 days (late phase) after STZ injection. The MSC-treated rats at 7 days were then infused with the same dose of MSCs at 42 days post-STZ administration. The untreated control T2D rats were infused with 0.2 mL physiological saline.

MSC homing and histopathologic analyses. MSCs were labeled 2 h prior to infusion with 2 μmol/L chloromethyl-benzamidodialkylcarbocyanine (CM-DiL; Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. On days 1, 3, and 7 post-MSC infusion, which was performed either during the early phase (day 7) or late phase (day 21) after STZ administration, animals were executed and their liver and pancreas were removed for preparation of cryosections. Labeled MSCs were calculated in 10 random fields of each of five tissue sections under fluorescence microscopy with 570-nm filters. Paraffin sections of liver and pancreas were stained with hematoxylin-eosin and used for histopathologic analysis.

Determination of the effect of infused MSCs on hyperglycemia in T2D rats. One week after STZ injection, oral glucose tolerance tests (OGTTs), intraperitoneal insulin tolerance tests (IPITTs), and insulin release tests (IRTs) were performed to confirm the T2D rat model. Two weeks after MSC infusion, OGTTs, IPITTs, serum insulin, and C-peptide were assessed again. Plasma glucose levels were monitored throughout the experiments with a glucometer-ACCU-CHEK Advantage Meter (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin and C-peptide measurements were performed by ELISA (Rat Insulin/C-peptide Elisa Kit; Millipore, St. Charles, MO).

Analytical methods and calculations for insulin resistance. Fasting blood glucose (FBG) and serum insulin (FINS) concentrations were measured in blood collected through tail prick at 2 weeks after MSC infusion. The homeostatic model assessment (HOMA) described previously was used to assess changes in insulin resistance (HOMA-IR) and pancreatic β-cell function (HOMA-β) in treated groups during the experimental period (22). The following equations were used to calculate the HOMA-IR index and HOMA-β index (HBCI): HOMA-IR index = (FBG [in mmol/L] × FINS [in units/L])/22.5 and HOMA-β = (20 × FINS [in units/L])/(FBG [in mmol/L] − 3.5).

Hyperinsulinemic-euglycemic clamp studies to assess insulin sensitivity. To define the role of MSCs in modulating insulin sensitivity, we performed hyperinsulinemic-euglycemic clamp studies, as described previously (23), at 2 weeks after MSC infusion. Blood glucose concentrations were monitored at 10-min intervals throughout the clamp studies. Basal and clamp hepatic glucose production (HGP), glucose disposal rate (GDR), and glucose infusion rate (GIR) were calculated as described previously (23,24). After the clamp studies, the rats were killed, and liver, epididymal adipose pads, muscle, and pancreas were removed for histological analysis.

Double-label immunohistofluorescence of the pancreas. Pancreatic sections were incubated with mouse monoclonal anti-rat insulin, rabbit anti-rat glucagons (Sigma-Aldrich), or rabbit anti-rat Ki67 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) (1:1,000) for 2 h. The sections were then washed with PBS with Tween 20 and incubated with Alexa Fluor 594–conjugated anti-mouse IgG and Alexa Fluor 488–conjugated anti-rabbit IgG (Invitrogen) for 1 h. Subsequently, pancreatic cryosections were stained with DAPI (Vector, Burlingame, CA) at 0.1 μg/mL. Sections were observed by fluorescence microscopy with 420- and 590-nm filters.

Western blot analysis. Total protein was extracted from samples of skeletal muscle, adipose tissue, and liver by PRO-PREP Protein Extraction Kit (iNtRON Biotechnology, Kyungki-Do, Korea). Aliquots containing 50 μg of protein were separated by 8% SDS-PAGE. Western blot analyses were performed using the monoclonal antibodies against GLUT2, GLUT4, total or phosphorylated IRS-1 (Tyr^{612}), and Akt (Ser^{473}) (Santa Cruz Biotechnology, Inc.), respectively. Anti-rat β-actin was used as a control (Sigma-Aldrich).

Plasma membrane protein preparations from skeletal muscle, adipose tissues, and liver were performed using Sigma-Aldrich Proteo-Prep Membrane Extraction Kit. Aliquots containing 25 μg of plasma membrane protein were subjected to 10% SDS-PAGE, and Western blot analysis was performed as described above. Anti–Na^+-K^+-ATPase α1-subunit (membrane marker) antibody was used as a control (Santa Cruz Biotechnology, Inc.).

Statistical analysis. All data are presented as means ± SE from at least four independent experiments. Statistical analysis was performed using SPSS version 14.0.1 for Windows. Individual data in experimental groups were compared with Student t test. Multiple comparisons were performed by ANOVA. Group differences at the level of $P < 0.05$ were considered statistically significant.

RESULTS

Characteristics of the fat-fed/STZ-induced T2D rat model. Success of fat-fed, STZ-induced T2D rat model was confirmed by checking for blood glucose, OGTTs, IPITTs, and IRTs, respectively. One week after STZ injection, blood glucose levels in the STZ-treated group increased to more than double that of normal rat (Supplementary Fig. 1*A*). The results of OGTTs revealed significant deterioration in glucose metabolism, and IPITTs showed a significant decrease in insulin sensitivity (Supplementary Fig. 1*B* and *C*). In IRTs, insulin secretion curves in response to glucose load in STZ-treated rats were consistent with that of T2D (Supplementary Fig. 1*D*). Moreover, morphological destruction of pancreatic islets characterized by significant reduction of islet size and decrease of insulin-positive cells was observed in STZ-induced rats (Supplementary Fig. 1*E*). Consistent with previous reports, these fat-fed, STZ-induced diabetic rats could be considered to be equivalent to stage 2 of diabetes (19,25).

Identification of BM-MSC characteristics. To identify MSC characteristics, phenotypes and multiple differentiating capacities of cultured BM-derived adherent cells at passage three were analyzed, respectively. As shown in Supplementary Fig. 2*A*, 95% of these cells were positive for CD29, CD44, and CD54, but negative for CD14, CD34, and CD45. They were also capable of differentiating toward osteogenic, chondrogenic, and adipogenic lineages under appropriate conditions as previously described (Supplementary Fig. 2*B*). These results indicate that the cultured cells possessed the characteristics of MSCs described in previous studies (20,21).

MSC infusion ameliorated hyperglycemia in T2D rats. During the study period, untreated T2D rats showed persistent hyperglycemia, combined with gradual decrease in body weight and higher mortality after fasting. Nevertheless, MSC-treated T2D rats showed a transiently significant and subsequently moderate decrease of blood glucose level (Figs. 1*A* and 2*A*) and significant improvement of blood glucose metabolism (Figs. 1*B* and 2*B*) and insulin sensitivity (Figs. 1*C* and 2*C*). These results together demonstrated that MSC administration could contribute to amelioration of hyperglycemia in T2D rats, whether infused during the early (day 7) or late phase (day 21) after STZ injection. However, the reduction in blood glucose levels caused by a single MSC infusion was only maintained for ~4 weeks. We then performed a second MSC infusion 42 days after the first infusion. As shown in Fig. 1*A*, blood glucose levels declined again, and reached similar lower values as observed after the first MSC administration. This response curve of blood glucose indicated that it is hard to maintain a long-term blood glucose

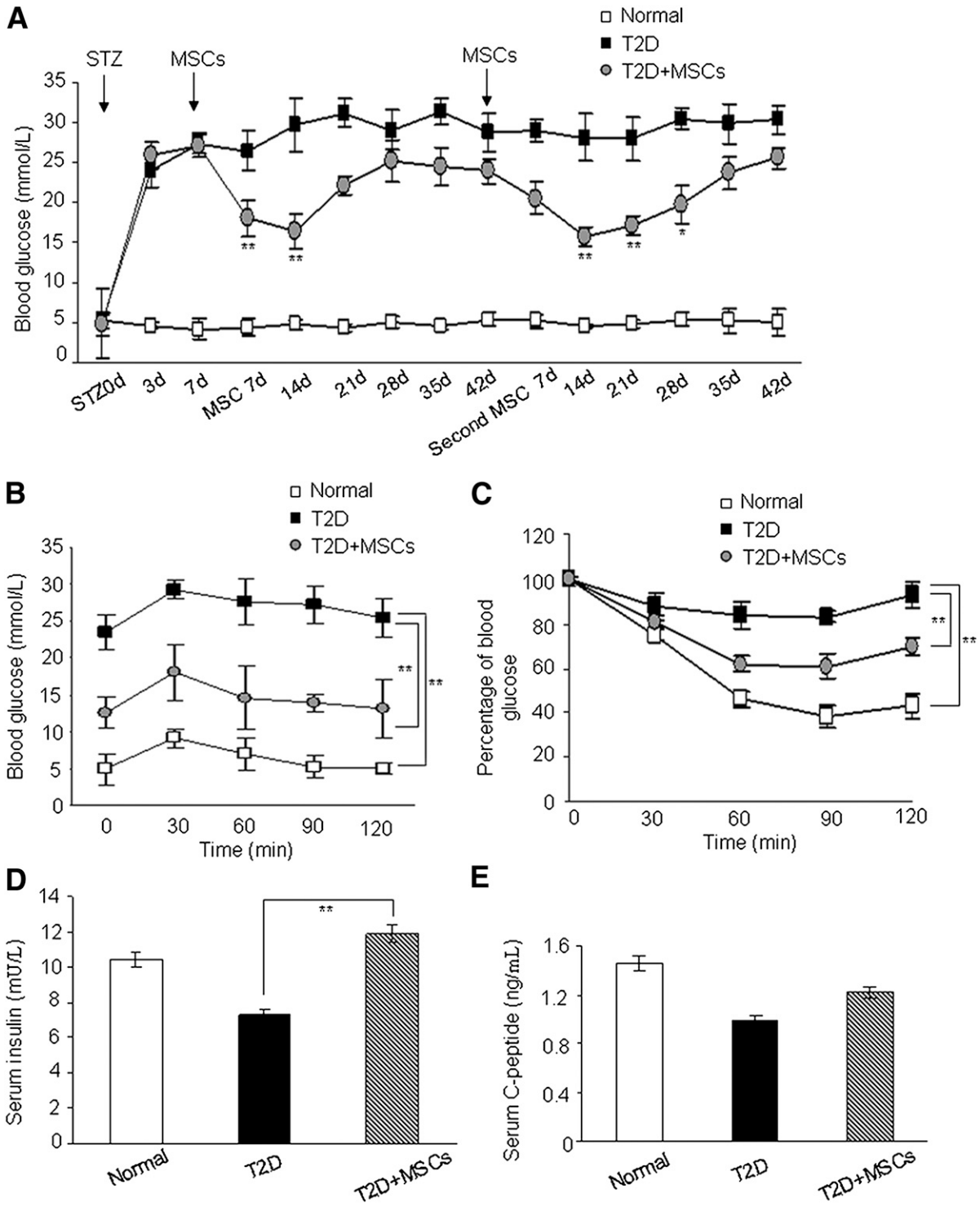

FIG. 1. Infusion of MSCs ameliorates hyperglycemia in T2D rats in the early phase (7 days) and 42 days after STZ injection, respectively. Seven days after STZ injection, diabetic rats received 0.2 mL physiological saline or 2×10^6 MSCs resuspended in 0.2 mL physiological saline, and the second MSC infusion was performed at 42 days after STZ injection. *A*: Blood glucose level was determined consecutively in alert, fasted rats using a glucometer-ACCU-CHEK Advantage Meter. d, day. Two weeks after MSC infusion, individual oral glucose tolerance was assessed by OGTTs, by intragastrically administering 2 mg glucose/g body weight and determining blood glucose levels (*B*); and insulin tolerance was evaluated by IPITTs, by injecting 2 g glucose/kg body weight immediately followed by insulin administration at a dose of 2 units/kg body weight (*C*). *D*: Individual insulin level in fasted and refed rats was evaluated by ELISA. *E*: Individual C-peptide level in fasted and refed rats was evaluated by ELISA. Values of *A–E* are means ± SE. n = 10 rats per group. *$P < 0.05$ and **$P < 0.01$.

decline in T2D rats with a single-dose MSC infusion. The limited role of single-dose MSC infusion on hyperglycemia is observed in most cases in diabetic animal models (5,14,26,27).

Accordingly, serum insulin concentration was examined at different time points throughout the study. Consistent with previous reports (19,25), at 7 days after STZ injection, T2D rats did not exhibit severe serum insulin deficiency

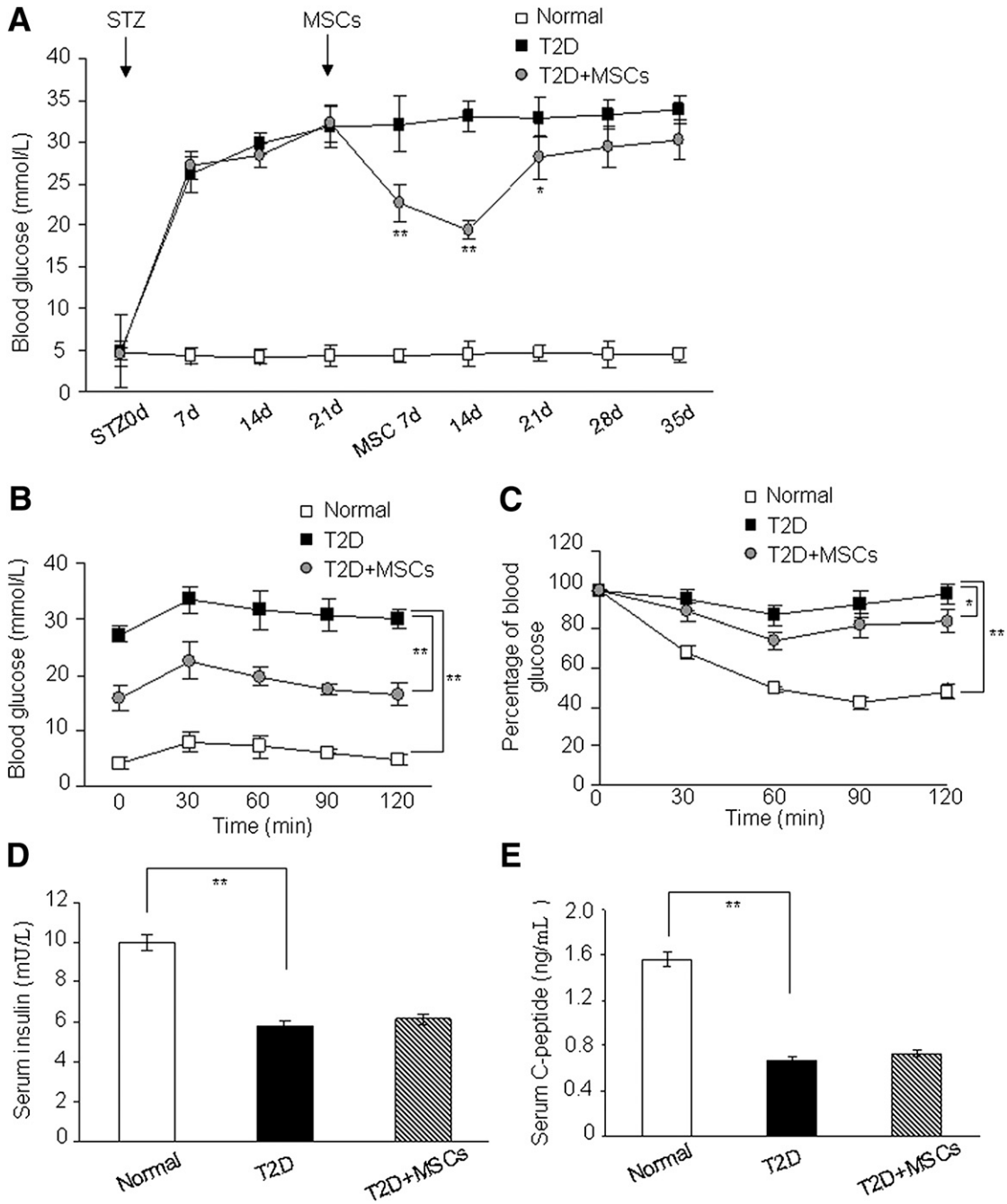

FIG. 2. Infusion of MSCs ameliorates hyperglycemia in T2D rats during the late phase after STZ injection. Twenty one days after STZ injection, diabetic rats received 0.2 mL physiological saline or 2 × 10^6 MSCs resuspended in 0.2 mL physiological saline. *A*: Blood glucose level was determined consecutively in alert, fasted rats using a glucometer-ACCU-CHEK Advantage Meter. d, day. Two weeks after MSC infusion, individual glucose tolerance was assessed by OGTTs (*B*), and individual insulin tolerance was evaluated by IPITTs (*C*). *D*: Individual insulin level in fasted and refed rats was evaluated by ELISA. *E*: Individual C-peptide level in fasted and refed rats was evaluated by ELISA. Values of *A–E* are means ± SE. *n* = 10 rats per group. **P* < 0.05 and ***P* < 0.01.

compared with normal rats (Supplementary Fig. 1*D*). In contrast, at 14 days after STZ injection, the marked decrease of insulin concentration in T2D can be observed (Fig. 1*D*). It indicated that an HFD combined with STZ administration induced progressive β-cell dysfunction in T2D rats. It was notable that MSCs infused during the early phase after STZ injection could induce a significant increase in insulin concentration (Fig. 1*D*), but not during the late phase (Fig. 2*D*). The trend of serum C-peptide variation was similar to that of insulin concentration (Figs. 1*E* and 2*E*).

Infusion of MSCs during the early phase promoted the recovery of STZ-induced liver and pancreas damage. To assess the influence of MSC treatment on STZ-induced tissue damage, we performed histopathologic analysis. As previously reported (25,28), HFD alone induced fat deposition, steatosis in liver, and hypertrophic islets in pancreas. HFD plus STZ induced an inflammatory reaction, necrosis or fibrosis in the liver, and decreased mass of pancreatic islets (Supplementary Fig. 3*A*). However, these pathological damages were effectively relieved by MSC

infusion during the early phase after STZ treatment, but not during the late phase (Fig. 3*A* and Supplementary Fig. 3*A*). Results from immunofluorescence staining showed that MSC infusion during the early phase ameliorated the destruction of pancreatic islets and produced a similar morphology to normal islets, which were organized with insulin-producing cells located centrally and glucagon-producing cells located peripherally (Fig. 3*B*). Nevertheless, MSC infusion during the late phase did not exhibit this apparent amelioration (Fig. 3*C*). Consistent with these results, MSC infusion during the early phase led to significant restoration of the numbers of pancreatic islets and the ratio of β-cells per islet (Fig. 3*D* and *F*). In contrast, MSC infusion during the late phase did not exhibit this effect (Fig. 3*E* and *G*). We next performed insulin/Ki67 double immunofluorescence to examine whether MSC infusion during the early phase could promote β-cell proliferation. However, Ki67-positive β-cells could not be detected in both MSC-treated and control groups (data not shown), suggesting that the "increased" islet and β-cell numbers were not generated by cell proliferation. These results suggested that only infusion of MSCs during the early phase could obviously relieve STZ-induced tissue damage in T2D rats, but not during the late phase. We also proposed that the significant restoration of islet number and β-cell "increase" upon MSC infusion during the acute phase of the T2D rat model may be largely caused by the cytoprotective properties of infused MSCs.

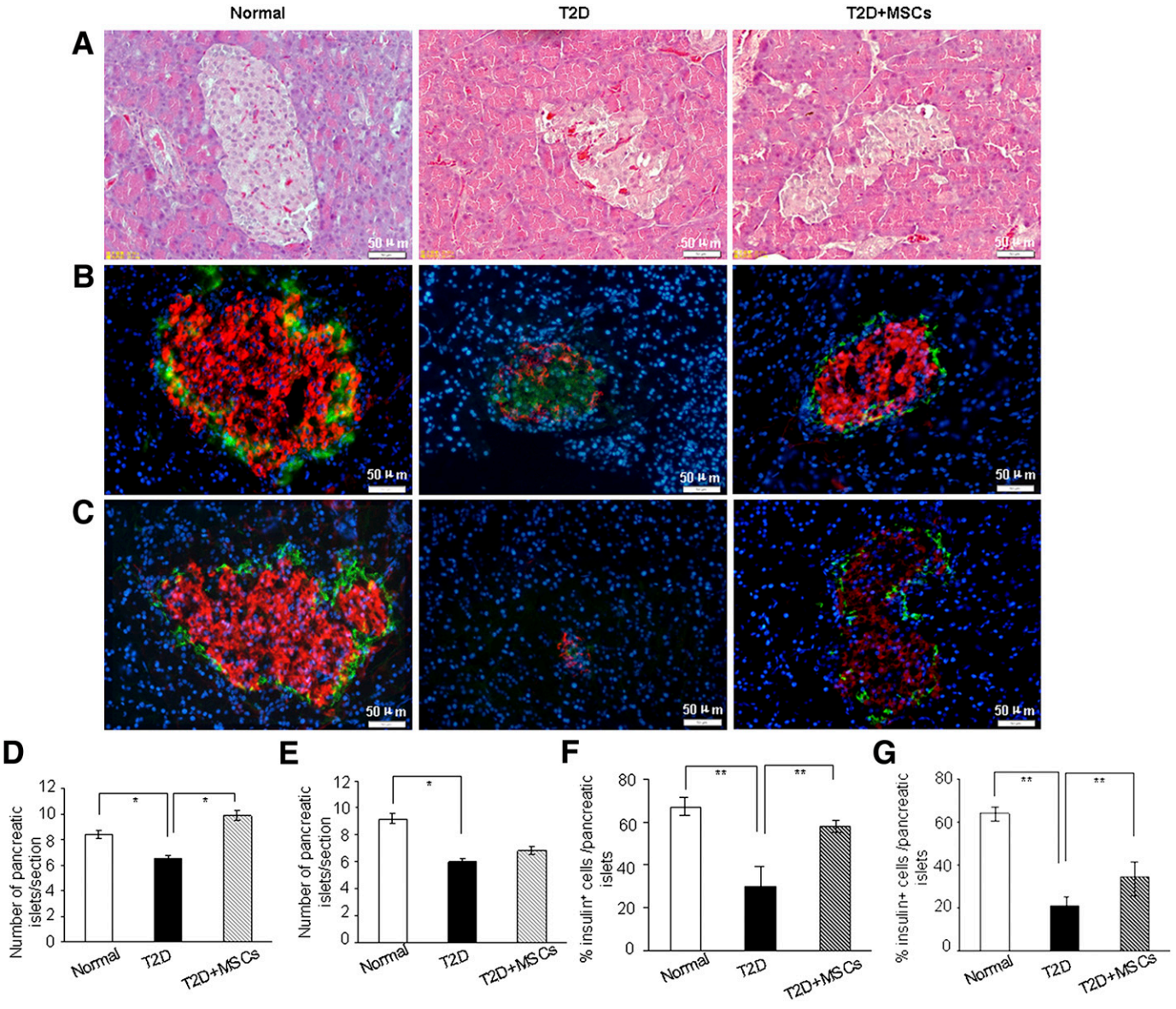

FIG. 3. Infusion of MSCs promotes restoration of pancreatic islet function in T2D rats. *A*: Pancreas histology was studied in hematoxylin/eosin-stained sections, observed under light microscopy and focusing on islet structures indicated by arrows. Pancreatic islets were characterized by immunofluorescence according to the presence and distribution of insulin- (red) and glucagon-producing (green) cells in the T2D rats that received MSC infusion at 7 (*B*) or 21 days (*C*) after STZ injection. Pancreatic islets observed in hematoxylin/eosin-stained sections were quantified in the T2D rats that received MSC infusion at 7 (*D*) or 21 days (*E*) after STZ injection. β-Cells in pancreatic islets were quantified in the T2D and MSC-treated T2D rats that received MSC infusion at 7 (*F*) or 21 days (*G*) after STZ injection. Images were composite overlay of the individually stained nuclei, insulin and glucagon from the continuous pancreatic cryosections. Scale bar, 50 μm (*A–C*). Values of *D–G* are means ± SE. $n = 5$ sections per group. *$P < 0.05$ and **$P < 0.01$. (A high-quality digital representation of this figure is available in the online issue.)

Several reports demonstrated that the pro-repair function of MSCs can be strengthened by their preferential homing to the acute injury tissues (29,30). We measured the homing status of infused MSCs in liver and pancreas at different time points. The results showed that MSCs infused during the early phase could preferentially home to the damaged tissues, but not when MSCs were infused during the late phase (Supplementary Fig. 3*C* and *D*). The increased recruitment of MSCs infused during the early-injured phase in damaged tissues suggests their contribution, at least in part, to relieving STZ-induced tissue damage, and subsequently to improving the overall metabolic outcome.

MSC infusion improved insulin sensitivity. Hyperinsulinemic-euglycemic clamp studies performed under euglycemic conditions (Supplementary Fig. 4*A*) showed the comparison of basal/clamp HGP, GDR, and GIR in each group (Fig. 4). As shown in Fig. 4*A* and *B*, GDR and GIR (GIR levels at the different time points were shown in Supplementary Fig. 4*B*) were significantly increased in MSC-treated diabetic rats. There was no significant difference in basal HGP between MSC-treated and untreated T2D rats, but clamp HGP was significantly lower in MSC-treated rats (Fig. 4*C* and *D*). Furthermore, the values of the IR index and HBCI in MSC-treated rats also reflected their reduced insulin resistance and improved β-cell function (Fig. 4*E* and *F*). Thus, these results together demonstrated that MSC infusion could improve insulin sensitivity, whether the infusion was performed during the early or late phase after STZ injection.

MSC administration influenced the expression and redistribution of GLUT4 in insulin target tissues. To determine the possible mechanism involved in the effect of MSC infusion on insulin resistance, we assessed the expression and redistribution of GLUT4 in skeletal muscle

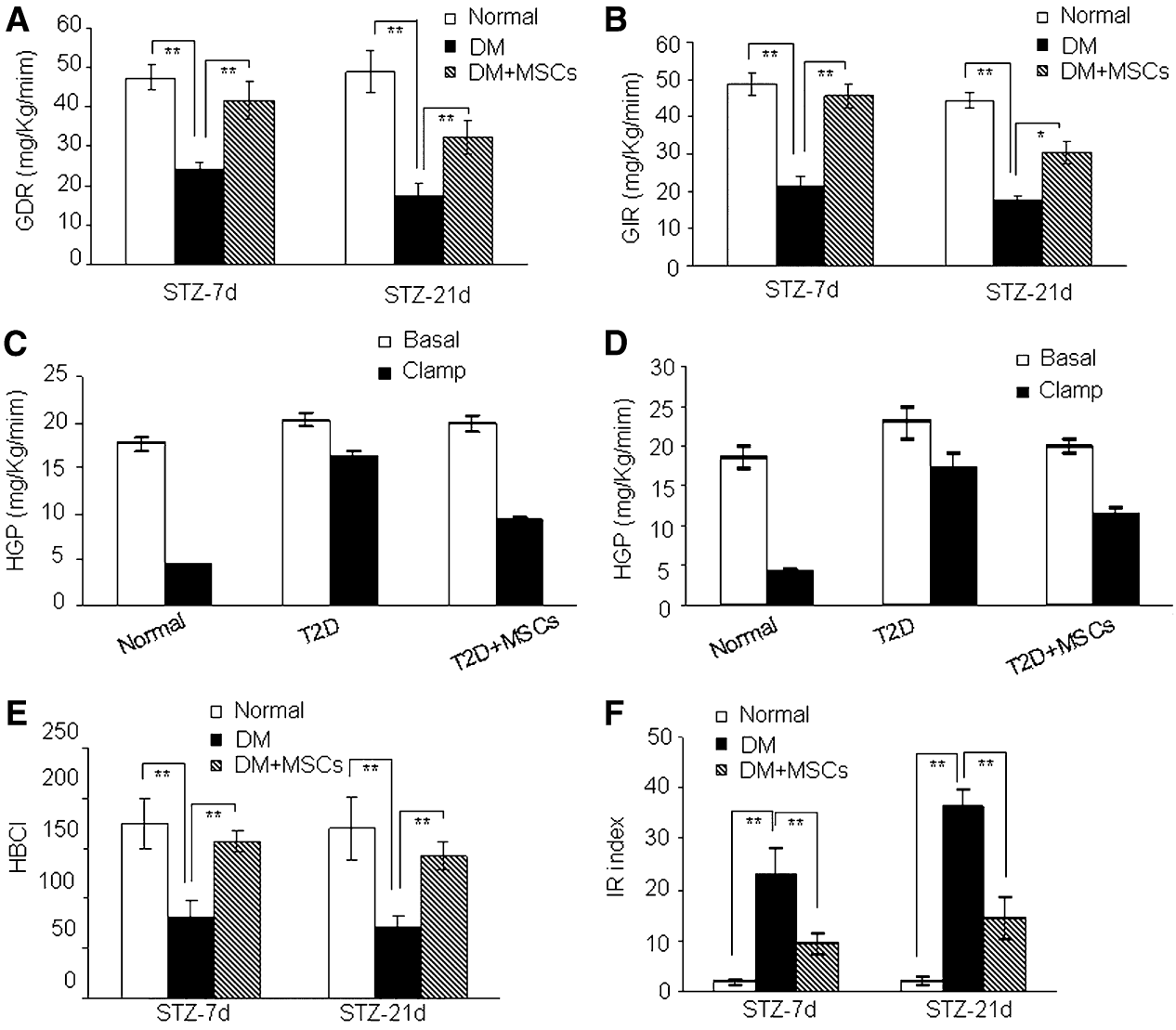

FIG. 4. Infusion of MSCs improved insulin sensitivity in T2D rats that received infusion at 7 or 21 days after STZ injection. *A* and *B*: Insulin sensitivity of each group was measured by euglycemic-hyperinsulinemic clamp. *A*: Exogenous GIR. All rats were infused with 8 mU/kg/min insulin during the hyperinsulinemic clamp. *B*: GDR of each group at insulin infusion rates of 8 mU/kg/min. *C* and *D*: HGP of each group at insulin infusion rates of 8 mU/kg/min, in the experiment in which MSC infusion was performed at 7 (*C*) or 21 days (*D*) after STZ injection. *E* and *F*: FBG and FINS concentrations of all rat groups were measured in blood collected at 2 weeks postinfusion by tail prick. *E*: HBCI of each group, HOMA-β (HBCI) = (20 × FINS [in units/L])/(FBG [in mmol/L] − 3.5). *F*: IR index of each group, HOMA-IR index = (FBG [in mmol/L] × FINS [in units/L])/22.5. Values of *A–F* are means ± SE. *n* = 10 rats per group. **P* < 0.05 and ***P* < 0.01. d, day. DM, diabetes.

and adipose and liver tissues during the late phase after STZ injection. The total and the plasma membrane fraction of GLUT4 in skeletal muscle and adipose tissues were dramatically decreased by STZ; however, both of them were partially restored by MSC administration. Although the total GLUT4 expression in liver tissue was also markedly increased after MSC treatment, the obvious increase of GLUT4 in the plasma membrane fraction was not observed (Fig. 5*A* and *B*). Thus, these results suggest that MSCs might mediate GLUT4 expression and translocation through a tissue-specific pathway and accordingly improve the sensitivity of target tissues to insulin action.

To further determine the possible regulatory role of MSC treatment on other GLUT family proteins, we performed immunoblotting in the livers of MSC-treated rats to measure the expression of GLUT2, a glucose-sensitive transporter. Consistent with previous studies (31), HFD treatment alone did not influence the expression of GLUT2, whereas HFD plus STZ treatment led to a significant increase of GLUT2. However, this STZ-induced elevation was partially reversed by MSC administration (Supplementary Fig. 5). These results showed the obviously inversed roles of MSC infusion on the expression levels of GLUT4 and GLUT2. Given that the expression of GLUT2 is inversely related to blood glucose level (32), it is reasonable to postulate that the downregulation of GLUT2 expression in T2D rat liver may be caused by the amelioration of hyperglycemia by infusion of MSCs.

MSC administration enhanced the phosphorylation of IRS-1 and Akt in T2D rat. The inability of feeding to induce the elevation of phosphorylated IRS-1 and Akt is currently believed to be a hallmark of insulin insensitivity or resistance in T2D animal models (33). To investigate the possible influence of MSC treatment on the IRS-1 and Akt phosphorylation level, we performed immunoblotting assays. As shown in Fig. 6, feeding induced significant increases of both phosphorylated IRS-1 and Akt levels in skeletal muscle and liver and adipose tissues of the control group. Compared with controls, marked impairment of the feeding-induced increase of phosphorylated IRS-1 and Akt in T2D rats was observed; however, this impairment can be partially suppressed by MSC infusion during the late phase after STZ treatment. These data suggest that MSC infusion can effectively potentiate the transduction of insulin signaling in insulin target tissues.

DISCUSSION

A number of previous studies and clinical trials have revealed that MSCs are capable of reducing glucose levels in animals or subjects with type 1 and type 2 diabetes (5,12,13). Based on current knowledge, it was considered that the underlying mechanism of the therapeutic effect of MSCs on hyperglycemia might involve islet regeneration, including direct differentiation into functionally competent β-cells (16,34–37). However, some controversial studies have suggested that the limited number of MSC-derived functional β-cells in vivo and the small amount of insulin produced by these cells seemed to be inadequate to maintain euglycemia (7,38,39). To explore these outstanding concerns, we induced T2D in a rat model by the method described previously (19) and performed MSC infusions during different phases after STZ injection. Consistent with other reports (5,25,34), our results proved that MSC infusion could partially reconstruct islet function and effectively ameliorate hyperglycemia in T2D rats.

Nevertheless, the beneficial effect of a single infusion of MSCs in ameliorating hyperglycemia in diabetic rats was maintained only for a period not exceeding 4 weeks. This rapid and relatively temporary effect of MSC infusion in ameliorating hyperglycemia could not be adequately explained by the partial restoration of islet function. Therefore, there might be other possible mechanisms underlying the effect of MSCs in lowering blood glucose levels in T2D.

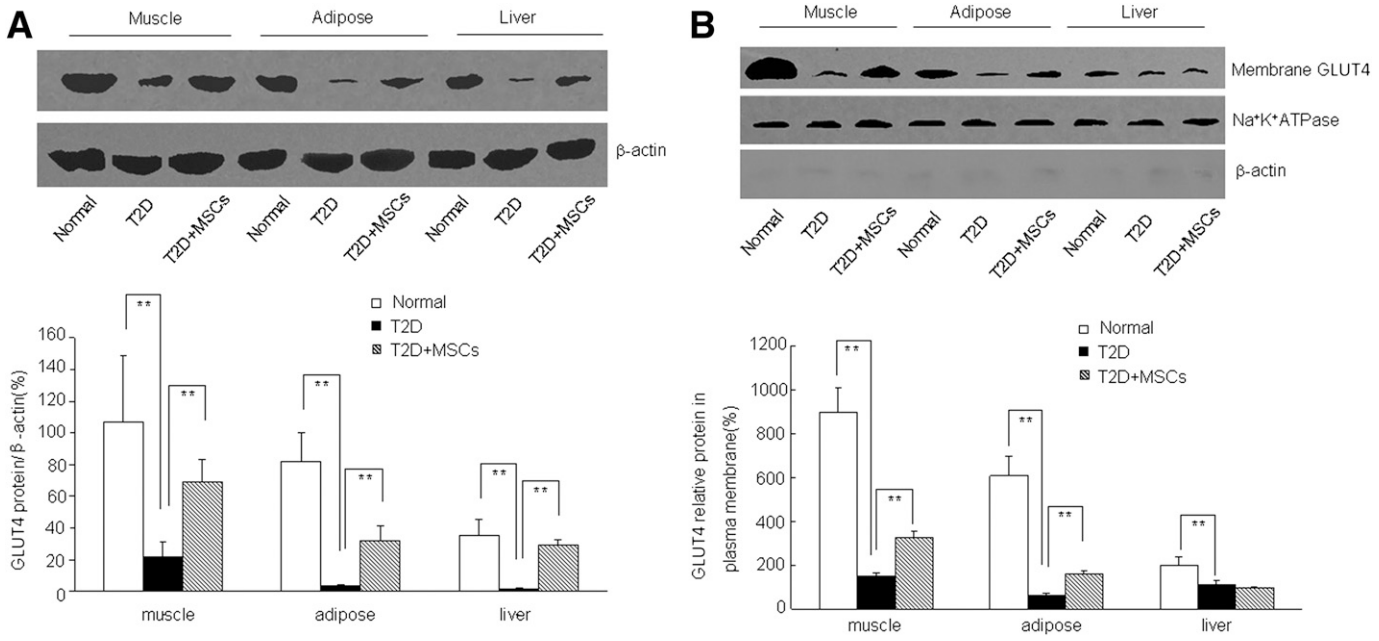

FIG. 5. Infusion of MSCs increased the expression and membrane translocation of GLUT4 in skeletal muscle, adipose tissue, and liver. *A*: Total GLUT4 expression in muscle, adipose tissue, and liver and quantitative analysis of relative GLUT4 levels normalized to β-actin. *B*: Membrane translocation of GLUT4 and quantitative analyses of relative membrane GLUT4 levels normalized to Na^+-K^+-ATPase. Values are means ± SE. *n* = 10 rats per group. **$P < 0.01$.

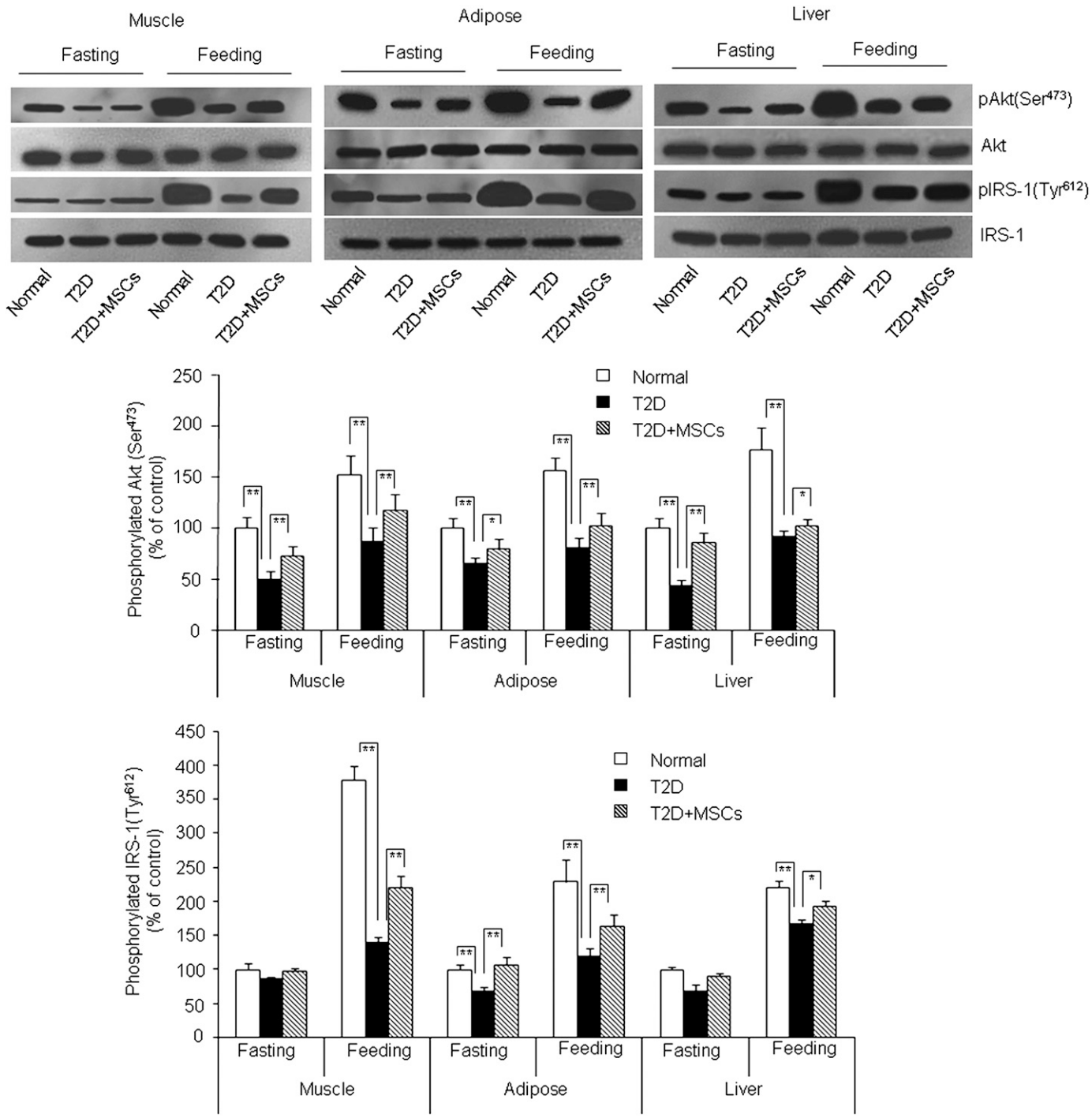

FIG. 6. MSC administration increased feeding-induced elevation of phosphorylated IRS-1 and Akt. The phosphorylated IRS-1 (Tyr^{612}) and Akt (Ser^{473}) in the indicated tissues were measured by immunoblotting and were normalized by total IRS-1 and Akt, respectively. The normalized values in fasting normal groups were arbitrarily designated as 100%. Values are means ± SE. n = 10 rats per group. *P < 0.05 and **P < 0.01.

Consistent with the notion that infused MSCs possess tissue repair and/or cytoprotective properties possibly due to their preferential homing property to acute-injured tissues (39), we observed the increased recruitment of MSCs within STZ-damaged tissues (liver and pancreas) and the corresponding tissue repair, including significant islet reconstruction, when MSCs were infused during the early injury phase (day 7), but not during the late phase (day 21). Thus, the remarkable tissue repair and the possible cytoprotective properties of MSC infusion should contribute to ameliorating hyperglycemia in the early phase of the acute-induced T2D rat model, but not in the late phase. However, given that the physiopathologic changes during the late phase of the T2D rat model that we used are more similar to T2D in humans compared with those during the early phase, we proposed that the observed effects of MSC infusion in the late phase would provide more helpful insight into the clinical situation.

Given the important role of insulin resistance in the development of T2D, it is doubtful whether MSCs could modulate insulin sensitivity of peripheral target tissues. To date, there have been no reports on this issue. To evaluate the possible effect of MSCs on insulin resistance in T2D rats, we established a T2D rat model with markedly decreased insulin sensitivity, as previously reported (40), and performed euglycemic-hyperinsulinemic clamp studies, which have become the standard reference method for the study of glucose metabolism. As demonstrated in Fig. 4, MSC treatment significantly improved insulin sensitivity, whether it was performed during the early phase or late phase after STZ injection. These findings demonstrate that MSCs can ameliorate hyperglycemia by modulating the insulin sensitivity of peripheral target tissues.

Based on the results mentioned above, multiple roles and mechanisms might be involved in the effects of MSCs on ameliorating hyperglycemia in T2D, including the promotion of β-cell function and amelioration of insulin resistance. Infusion of MSCs during the early phase of T2D was able to not only promote β-cell function but also ameliorate insulin resistance, whereas MSC infusion during the late phase merely ameliorated insulin resistance.

To date, the molecular mechanism of insulin resistance in diabetes has been explored extensively. GLUT4 translocation from intracellular vesicles to the plasma membrane is considered the terminal step of insulin signal transduction. Reduced amounts of GLUT4 protein and reduced translocation of GLUT4 to the cell membrane due to insulin stimulation are implicated in insulin resistance and impaired glucose metabolism (41,42), whereas elevation of GLUT4 protein content and its translocation to the cell membrane of peripheral insulin target tissues are responsible for improvement in sensitivity to insulin action. Our study revealed that in skeletal muscle and adipose tissues, MSC infusion could restore the concentration of total GLUT4 and promote GLUT4 translocation to the cell membrane, thus facilitating glucose utilization to ameliorate insulin resistance. However, in liver tissue, MSCs increased total GLUT4 expression alone but did not influence the translocation of GLUT4.

Because MSC infusion into T2D rats during the early phase was able to increase the insulin secretion level, a question was raised as to whether MSCs improve insulin resistance through an insulin-dependent pathway or by directly affecting GLUT4. In a number of studies, insulin was found to dose-dependently increase the translocation of GLUT4 but could not influence the total protein expression level of GLUT4 in the state of induced insulin resistance (43). To investigate this question and avoid the possible influence of serum insulin concentration, we compared insulin level and GLUT4 expression in the insulin target tissues that were removed from each group during the late phase after STZ injection. Our results showed that although MSC infusion did not induce an increase in insulin concentration, the total amount of GLUT4 protein significantly increased compared with untreated T2D rats. Therefore, this demonstrates that MSCs were able to restore the expression of total GLUT4 protein through an insulin-independent pathway.

Functional membrane translocation from the cytoplasm of glucose transporters in insulin-responsive tissues is cardinally regulated by insulin signaling. Elevation of the cascaded phosphorylation of IRS-1 and Akt triggered by exogenous factors, including insulin, is essential for membrane translocation of GLUT4 (17,18,34). In this study, we observed that MSC treatment could not only increase the expression level of GLUT4 on the insulin target cell membrane of T2D rats but also partially restore the feeding-induced phosphorylation of IRS-1 and Akt. These findings suggest that the improvement of insulin sensitivity by MSC infusion may be virtually caused, at least partially, by the enhanced effect of MSCs on insulin signaling transduction. Certainly, it cannot be excluded that the MSC infusion–induced expression increase of GLUT4 may, to some extent, compensate the blunted insulin signaling in T2D models. The precise mechanisms by which MSC infusion regulates GLUT4 expression and IRS-1/Akt phosphorylation remain to be determined.

In summary, we verified that multiple roles and mechanisms were involved in the effect of MSCs in ameliorating hyperglycemia in T2D, and this is the first report of a novel role of MSC administration in improving insulin sensitivity. In addition, this is the first study to show that MSC infusion lead to increased GLUT4 expression and the member translocation in peripheral insulin target tissue through an insulin-independent manner. These novel findings established an important foundation for exploring MSC infusion to improve insulin sensitivity in T2D therapy.

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Y.S. designed and conducted the experiments, performed data analyses, and wrote the manuscript. Y.Z., H.H., and Y.M. designed and conducted the experiments. J.L., J.S., and Y.C. conducted the experiments and analyzed data. Y.G. conducted glucose-clamp experiments and analyzed data. X.F. contributed to the review of the manuscript. W.H. contributed to the design and performance of the experiments and reviewed and revised the manuscript. W.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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