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Original Research

Can Stem Cells Ameliorate the Pancreatic Damage Induced by Streptozotocin in Rats?

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Key Messages

- Mesenchymal stem cell (MSC) and insulin-producing cell (IPC) therapy significantly improved several key indicators in the streptozotocin-induced type I diabetes model.
- Bone marrow-derived MSCs have the capacity to differentiate into IPCs capable of controlling the blood glucose level in rats with streptozotocin-induced diabetes.
- Stem cell therapy holds a great promise for the repair of injured tissues and organs and it is one of the most promising therapies for diabetes mellitus.

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ABSTRACT

Background: Stem cell therapy holds great promise for the repair of injured tissues and organs, and it is one of the most promising therapies for diabetes mellitus. Therefore, the present study was undertaken to elucidate the antidiabetic effect of both mesenchymal stem cells (MSCs) and insulin-producing cells (IPCs) on streptozotocin (STZ)–induced diabetes in rats.
Materials and methods: MSCs were derived from bone marrow of male albino rats. MSCs were characterized morphologically and by Cluster of differentiation (CD^{-ve34}) and (CD^{+ve105}). They were then differentiated into IPCs, and both MSCs and IPCs were infused independently into tail veins of rats with STZ-induced diabetes.
Results: MSC and IPC therapy significantly improved the body weight and serum insulin, alpha-amylase, adiponectin, creatinine, total cholesterol, triacylglycerol, interleukin-6, tumour necrosis factor-alpha, liver L-malonaldehyde and glycogen levels in the STZ-induced diabetes model.
Conclusions: Bone marrow–derived MSCs have the capacity to differentiate into IPCs capable of controlling the blood glucose level in rats with STZ-induced diabetes. Furthermore, treatment with MSCs and IPCs can improve aberrant biochemical parameters in an STZ-induced diabetes model.
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Mots clés :
diabète
différenciation
cellules souches mésenchymateuses
thérapie par les cellules souches

RÉSUMÉ

Introduction : Riche de promesses dans la réparation des tissus et des organes lésés, la thérapie par les cellules souches est l'une des thérapies les plus prometteuses dans le traitement du diabète sucré. Par conséquent, la présente étude était entreprise pour élucider les effets antidiabétiques des cellules souches mésenchymateuses (CSM) et des cellules productrices d'insuline (CPI) sur le diabète induit par la streptozotocine (STZ) chez les rats.

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Matériaux et méthodes : Les CSM ont été dérivés de la moelle osseuse de rats mâles albinos. Les CSM ont été caractérisées de manière morphologique et par groupe de différenciation (CD^{-ve34} et CD^{+ve105}). Elles se sont ensuite différenciées en CPI, puis les CSM et les CPI ont été infusées de façon indépendante dans les veines de la queue des rats ayant un diabète induit par la STZ.
Résultats : La thérapie par les CSM et les CPI a amélioré de manière significative le poids corporel et les concentrations sériques de l'insuline, de l'alpha-amylase, de l'adiponectine, de la créatinine, du cholestérol total, du triacylglycérol, de l'interleukine-6, du facteur de nécrose tumorale alpha, du L-malonaldéhyde hépatique et du glycogène dans le modèle de diabète induit par la STZ.
Conclusions : Les CSM dérivées de la moelle osseuse ont la capacité de se différencier en CPI capables de maîtriser la concentration de la glycémie chez les rats ayant un diabète induit par la STZ. De plus, la thérapie par les CSM et les CPI peut améliorer les valeurs aberrantes des paramètres biochimiques dans un modèle de diabète induit par la STZ.

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Introduction

The most common endocrine disorder worldwide is diabetes mellitus (DM), and it is usually accompanied by several complications, such as retinopathy, neuropathy, nephropathy and cardiovascular disease (1). It is associated with severe microvascular and macrovascular complications and is considered a major public health problem that may lead to global mortality (2). Oxidative stress, which causes an increase in free radical formation, plays an essential role in the development of DM and increases complications of diabetes (3). It also produces reactive oxygen species, resulting in a toxic effect on cell evolution, growth and permanence (4). Stem cell therapy has created unbelievable benefits for tissue and organ rebuilding and restoration (5). Because transplanted islet tissue very closely mimics the physiology of the wasted islets, patients no longer require daily insulin injections. Transplantation is limited by the equipping and viability of islet cells (6). Stem cells can ameliorate the equipping of pancreatic islet cells (7). They can be differentiated into beta cells to increase beta-cell supplies, enhance the islet microenvironment to support beta-cell function and survival in vivo (8). Treatment of bone marrow–derived mesenchymal stem cells (BMSCs) with insulin-promoting factors, such as nicotinamide, high glucose induction and growth factors, such as activin A and glucagon-like peptide 1, differentiates them into insulin-producing cells (IPCs). Nicotinamide is a well-known inducer for differentiating stem cells into IPCs and protects cells from glucotoxicity induced by exposure to high glucose concentrations (9). A high glucose concentration is considered a potent inducer of pancreatic islet cell differentiation (10). Moreover, activin A is a member of the transforming growth factor-beta–regulated neogenesis of beta cells in vivo (11). They are of particular interest because patients with DM can experience improved beta-cell function (8). Therefore, the present study was designed to evaluate the potential roles of both mesenchymal stem cell (MSCs) and IPCs in controlling damage to vital tissues in streptozotocin (STZ)-induced diabetes in rats.

Materials and Methods

Preparation of BMSCs

Bone marrow was isolated and differentiated according to the method of Tariq et al (12) with some modification. Bone marrow was harvested by flushing the tibiae and femurs of 3- to 4-month-old male albino rats with Dulbecco's modified Eagle's medium (DMEM) (GIBCO/BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS) (GIBCO/BRL). Nucleated cells were isolated and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (GIBCO/BRL). The cells were incubated at 37°C in 5% humidified carbon dioxide for 12–14 days as a primary culture. When large colonies developed, the cultures were washed twice with phosphate-buffered saline (PBS), and the cells were trypsinized with 0.25% trypsin in 1 mmol/L ethylenediamine tetraacetic acid (EDTA) (GIBCO/BRL) for 5 minutes at 37° C. After centrifugation, the cells were resuspended in serum-supplemented medium and incubated in a 50-cm^2 culture flask. The resulting cultures were referred to as "first passage cultures" (13). MSCs in culture were characterized by their adhesiveness and fusiform shape (14) and also by Cluster of differentiation (CD^{-ve34}) and (CD^{+ve105}), which are surface markers of rat MSCs in culture. The BMSCs were differentiated into IPCs at third passage (70%–80% confluence). The cells were induced with DMEM-low glucose containing 0.5 mmol/L 2-mercaptoethanol, 10 mmol/L nicotinamide and 5% FBS for 2 days. The preinduced cells were further treated with serum-free DMEM high-glucose (DMEM-HG) medium containing 0.5 mol/L 2-mercaptoethanol, 10 mmol/L nicotinamide, 5% FBS and 10 ng/mL activin A for 24 hours. The cells were cultured for an additional 8 days in new DMEM-HG medium containing 20 ng/mL basic fibroblast growth factor, 20 ng/mL epidermal growth factor, 2 mmol/L L-glutamine, 5% FBS and10 mmol/L nicotinamide. After the 10th day of differentiation, cells were washed twice with PBS and trypsinized. After centrifugation at 2400 rpm for 10 minutes, cells were resuspended with serum-supplemented medium and incubated in a 50-cm^2 culture flask, counted by using a hemocytometer and identified by glucose challenge test (15).

Immune staining

The MSC phenotype was confirmed by immune staining using 2 markers known to be found on MSCs. The negative marker was anti-CD^{-34} (BD-Pharmingen, Palo Alto, California, United States), which is known to be expressed on hematopoietic stem cells and common lymphocytes. CD^{+105} was used as a positive marker for MSCs. This was determined by using Thermo Fisher Scientific kit (Thermo Fisher Scientific, Cheshire, UK), then the photograph was taken by a microscope (Carl Zeiss, Jena, Germany) with a digital camera (Canon-600; Canon Inc., Tokyo, Japan).

Principle

This ultravision detection system detects a specific antibody bound to an antigen in tissue sections. The specific antibody is located by a biotin-conjugated secondary antibody. This step is followed by the addition of streptavidin-enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody, secondary antibody and streptavidin-enzyme complex are then visualized with an appropriate substrate/chromogen.

Experimental animals

Eighty male albino Wistar rats, 3 to 4 months old with an average body weight of 180 to 220 g, were used in this study. Rats were purchased from the Institute of Ophthalmology, Nasser Eye Institute, Giza, Egypt. Animals were housed in a room under controlled

conditions (at a temperature of 32° with a 12-hour light-dark cycle and free access to water and chow [EL Haramen for poultry feeding, Quesna, EL Monfia]). Rats were handled according to the suggested National Ethical Guidelines for the Care of Laboratory Animals. as the Animal Ethics Committee of Faculty of Science, Tanta University, Egypt. The animals were left for 2 weeks for acclimatization before the beginning of the experiment.

Induction of diabetes

Rats were fasted for 18 hours and allowed free access to water. Type 1 diabetes was induced in each rat by a single intraperitoneal injection of STZ (40 mg/kg body weight), freshly dissolved in 0.2 mL of citrate buffer (0.1 mol/L, pH 4.5) (12). One week later, rats were fasted, and blood glucose levels were determined; the rats with blood glucose levels higher than 250 mg/dL were considered diabetic (16).

Experimental design

The rats were divided into the following 4 groups, each containing 20 rats.

Group I, the control group: Healthy rats received no drugs and served as the control group for all experiments.
Group II, STZ-induced diabetes group.
Group III, MSC-treated group: MSCs (1×10^5 cells/rat) were injected via tail veins (17) in rats with STZ-induced diabetes.
Group IV, IPC-treated group: IPCs (1×10^5 cells/rat) were injected via tail veins (17) in rats with STZ-induced diabetes.

Thirty-five days after induction of diabetes, treatment was started with both MSC and IPCs. Each of the 4 groups described previously was subdivided into 2 subgroups.

Sampling

At the end of the 4th and 6th weeks after initiation of treatment with MSCs and IPCs, random blood samples were collected by ocular vein puncture from rats in all groups after overnight fasting, and serum was separated by centrifugation at 3000 rpm for 15 min. The clean and clear serum samples were immediately processed for glucose and lipid profile determination and kept at –20° C until they were used for subsequent biochemical analysis. Liver and pancreatic tissue specimens were also collected.

Body weight

Body weights of the rats were determined at the end of the 4th and 6th weeks.

Biochemical parameters

Serum glucose was estimated enzymatically (18), using a kit obtained from Spectrum Diagnostics, (Obour City, Egypt). Serum insulin was measured with the use of an ultrasensitive rat insulin enzyme-linked immunoassay (ELISA) kit (Mercodia AB, Uppsala, Sweden). The serum glucose-to-insulin ratio was calculated according to the formula described by Silfen et al (19). The activity of serum alpha-amylase was estimated according to the method of Winn-Deen et al (20), and the level of adiponectin in the serum was determined by using an ELISA kit according to the manufacturer's instructions (BioVendor Laboratory Medicine, Inc, Brno, Czech Republic) and a multiplate ELISA reader (Biorad-680, Biorad Ltd, Hercules, California, USA) (21).

Determination of serum lipid profile

Serum total cholesterol and triacylglycerol were estimated enzymatically using a kit obtained from Spectrum Diagnostics. (22, 23).

Determination of serum kidney and liver function

Serum urea was evaluated enzymatically (24), and the serum creatinine concentration was determined by the kinetic method with a kit from Spectrum Diagnostics (25). Furthermore, both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined enzymatically according to the method described by Breuer (26) using reagent kits purchased from Spectrum Diagnostics.

Determination of serum proinflammatory markers

Determinations of serum interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α) were performed according to the methods of Ferrari et al (27) and Tian et al (28), respectively, using an ELISA kit from ALP CO Diagnostics (Salem, New Hampshire, United States).

Tissue samples (liver and pancreas)

An appropriate portion (10% wt/vol) of the liver was homogenized in 0.9% normal saline solution using a tissue Homogenizer, WiseTisHG-15A-Set Homogenizer (MegaLab S. A., Thessaloniki, Greece) (29). The homogenates were centrifuged at 4000 rpm for 20 minutes, and the supernatants were used for biochemical analysis. In addition, small pieces of pancreas tissue were obtained from all rats in the experimental groups and preserved in 10% buffered neutral formalin. The samples were then dehydrated, cleared and embedded in paraffin and sectioned at 5 µm. Some slices were dewaxed, rehydrated and processed for hematoxylin and eosin staining.

Determination of hepatic L-MDA and glycogen levels

Liver L-MDA levels were determined according to the method described by Mesbah et al (30). Also, 1 g of liver tissue was homogenized in potassium hydroxide and set to boil at 100°C for 30 minutes. The solution was treated with ethanol to precipitate glycogen. Absorbance was measured at 492 nm using a multiplate ELISA reader (31).

Statistical analysis

The data were statistically analyzed by one-way analysis of variance, followed by Duncan multiple tests. All analyses were performed using the Statistical Package for Social Science (CoHort/CoStat Software, Version 6.311, 2005). Probability values ≤0.05 were considered significant.

Results

MSC culture and identification

Isolated and cultured undifferentiated MSCs reached 70%–80% confluent after 14 days of culturing the cells. The confluence was be confirmed by examine under inverted microscope. MSCs were identified by their adhesiveness and fusiform shape and also by surface markers $CD34^{-ve}$ (Figure 1) and $CD105^{+ve}$ (Figure 2), which are detected by immune staining.

Glucose challenge test

IPCs were analyzed in vitro by ELISA after exposure to a high concentration of glucose (25 mmol/L). The differentiated cells secreted around 15.01±1.04 nIU/mL insulin at a high glucose level.

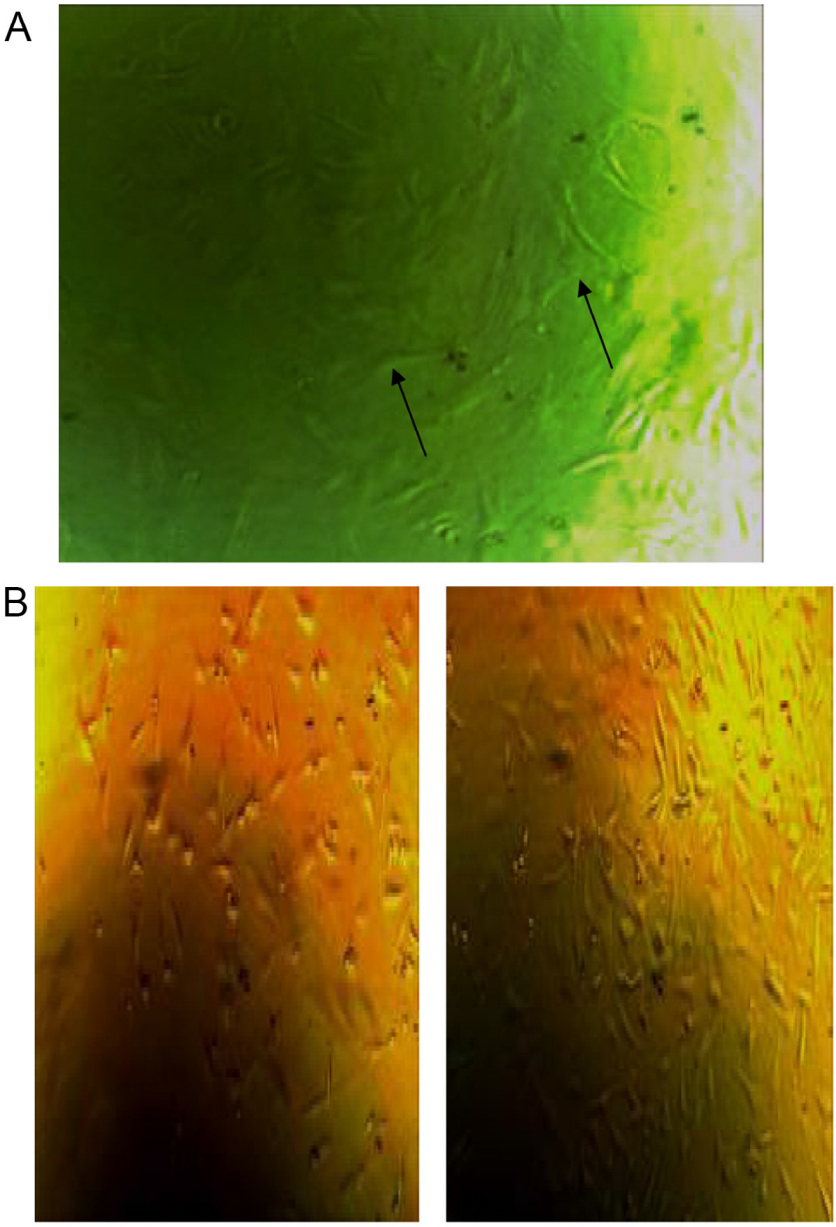

Figure 1. A, The morphology of mesenchymal stem cells from culture at 3 days old primary; B, The morphology of mesenchymal stem cells from culture colony 5 days old primary.

Effect of cell transplantation treatment on body weight

The changes in body weight in all experimental groups of rats are shown in Table 1. Body weight in the STZ-induced diabetes group (hereafter referred to as "the STZ group"), MSC-treated group and IPC-treated group was significantly reduced ($p<0.05$) when compared with the control group. However, after 6 weeks, there was a significant increase ($p<0.05$) in body weight in both the MSC-treated group and the IPC-treated group, but it did not reach the normal value as compared with the control group.

Effect of cell transplantation treatment on blood sugar, serum insulin, glucose-to-insulin ratio, α-amylase activity and serum adiponectin level in rats with STZ-induced diabetes

Both serum glucose and insulin levels were assessed to ensure the hyperglycemic state. The data in Tables 2 and 3 show an incredible increase ($p<0.05$) in the serum glucose levels in the STZ group and the MSC- and IPC-treated groups as compared with the control group. However, the MSC-treated group had significantly reduced ($p<0.05$) serum glucose levels after 6 weeks when compared with the STZ group, but it did not reach the normal value as compared with the control group. Significant decreases in serum insulin levels were observed in the STZ group, the MSC-treated group and the IPC-treated group as compared with the control group. Both the MSC-treated group and the IPC-treated group showed a significant increase ($p<0.05$) in serum insulin levels after 6 weeks of the experiment as compared with the STZ group, but it did not reach the normal value as compared with the control group. Furthermore, the serum glucose-to-insulin ratio showed a significant increase in the STZ group and the MSC-treated and IPC-treated groups after 4 weeks of the experiment as compared with the control group. However, both the MSC-treated group and the IPC-treated group had a significantly reduced serum glucose-to-insulin ratio

Table 1
Changes in the mean body weight in the different groups under study

Parameter	Time	Animal groups			
		I	II	III	IV
Body weight (g)	4 weeks	234.60±2.04[a]	186.00±2.81[b]	144.84±6.05[d]	160.30±4.49[c]
	6 weeks	249.80±1.562[a]	155.4±2.821[d]	187.48±9.867[b]	165.06±8.793[c]

Note: Group I (4 and 6 weeks) and group II (4 and 6 weeks) cited from El Barky et al (57).
Data are presented as (Mean ± S.E).
Mean values with different superscript letters in the same column are significantly different at ($p \leq 0.05$).
[a, b, c, d, e] Indicated the difference or similarity between experimental groups. Control normal group (Group I), diabetic group (Group II), diabetic MSCs (Group III) and diabetic IPCs (Group IV).

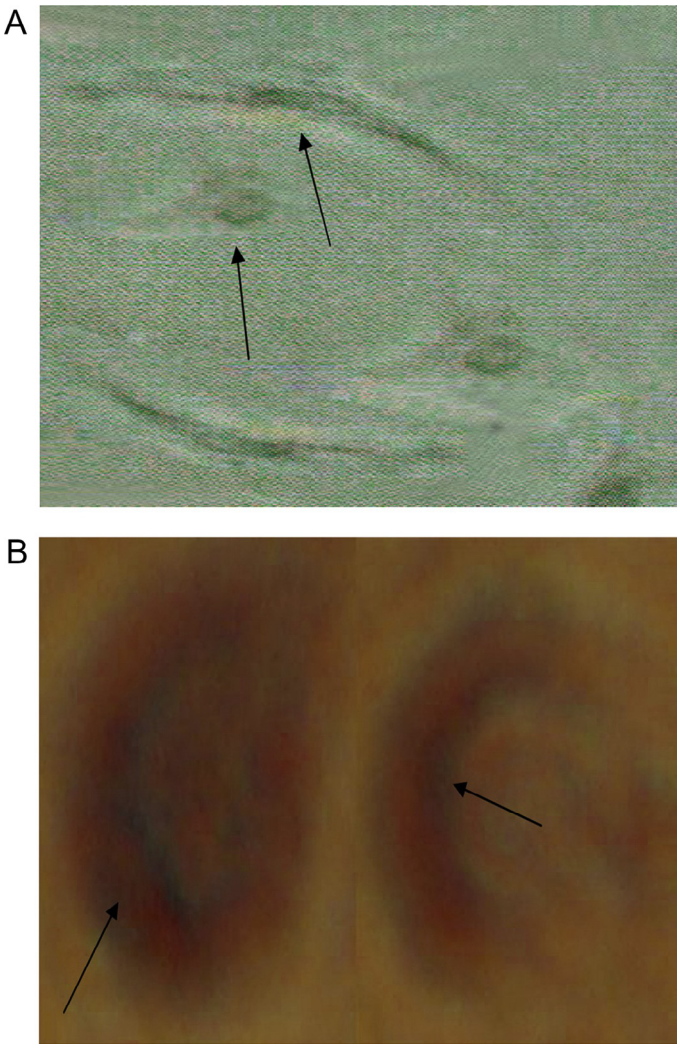

Figure 2. A, Photomicrograph showing CD34^{-ve}; photomicrograph showing CD105^{+ve}.

($p<0.05$) as compared with the STZ group, but it did not reach the normal value as compared with the control group. Also, the results pertaining to serum α-amylase activity showed a nonsignificant change after 4 weeks of the experiment in the STZ-induced diabetes group as compared with the control group. However, after 6 weeks, a significant increase in the activity of serum α-amylase ($p<0.05$) was observed in the STZ group as compared with the control group. Both MSC- and IPC-treated rats had a nonsignificant change in serum α-amylase activity after 4 weeks. Meanwhile, after 6 weeks, there was a significant decrease ($p<0.05$) of serum α-amylase activity as compared with that in the STZ group, and there was a nonsignificant change ($p<0.05$) as compared with the control group. In addition, the serum adiponectin concentration in the STZ group showed a significant increase ($p<0.05$) after 4 and 6 weeks of the experiment as compared with the control group. Meanwhile, both MSC- and IPC-treated rats showed a nonsignificant change ($p>0.05$) in serum adiponectin concentration after 4 weeks in comparison with the STZ group and showed a significant increase when compared with the control group. However, after 6 weeks, the MSC-treated group had significantly decreased serum adiponectin levels as compared with the STZ group, and serum adiponectin levels returned to normal values in comparison with the control group. In the IPC-treated group, serum adiponectin levels were not significantly reduced when compared with those in the diabetic group but were significantly increased when compared with those in the control group.

Effect of cell transplantation treatment on serum lipid profile parameters

Serum total cholesterol and triacylglycerol levels, which were detected in different treated groups, showed a significant increase ($p<0.05$) in both serum total cholesterol and triacylglycerol concentrations after 4 weeks in the STZ group. Meanwhile, a significant decrease ($p<0.05$) was observed in the STZ group after 6 weeks as compared with the control group. Conversely, both MSC-treated and IPC-treated groups showed a significant reduction in both serum total cholesterol and triacylglycerol concentrations after 4 weeks as compared with the STZ group, but they did not reach normal values as compared with the control group. The MSC-treated group had a nonsignificant change in serum cholesterol levels after 6 weeks as compared with the STZ group and showed a significant decrease ($p<0.05$) in comparison with the control group. The MSC-treated group had a significantly increased serum triacylglycerol level as compared with the STZ group, and it reached the normal value as compared with the control group. Although the IPC-treated group had significant increases in both serum cholesterol and triacylglycerol after 6 weeks as compared with the STZ group, the increases were not significant in comparison with the control group (Tables 2 and 3).

Efffect of cell transplantation treatment on kidney and liver function

Serum urea levels showed a significant increase ($p<0.05$) in the STZ group after 4 and 6 weeks as compared with those in the control group. Both MSC- and IPC-treated rats showed a nonsignificant change after 4 and 6 weeks as compared with rats in the STZ group and the control group. In addition, serum creatinine levels, which were detected in the different treated groups, showed a nonsignificant change ($p>0.05$) in all studied groups after 4 weeks of the experiment as compared with the control group. However, a significant increase ($p<0.05$) in serum creatinine levels in the STZ group was observed after 6 weeks when compared with the control group. Both MSC- and IPC-treated groups showed a significant reduction in serum creatinine levels ($p<0.05$) as compared with the STZ group and showed a nonsignificant change when compared with the

Table 2
Serum and liver biochemical parameters in the different groups under study with treatment duration of 4 weeks

Parameter	Animal groups			
	I	II	III	IV
Glucose (mmol/L)	3.84±0.10[b]	20.60±0.54[a]	20.94±0.23[a]	20.92±0.13[a]
Insulin (pmol/L)	0.42±0.00[a]	0.14±0.00[b]	0.14±0.00[b]	0.14±0.00[b]
Glucose-to-insulin ratio	1274.23±89.66[b]	17442.05±1448.57[a]	19149.65±1497.18[a]	22783.10±1660.95[a]
α-Amylase (U/L)	274.89±23.25[a]	296.32±16.47[a]	289.21±6.71[a]	298.72±2.43[a]
Adiponectin (pg/mL)	442.59±53.22[b]	804.76±31.78[a]	675.53±35.32[a]	789.84±44.02[a]
Cholesterol (mmol/L)	1.13±0.07[d]	2.99±0.05[a]	1.91±0.10[b]	1.55±0.13[c]
Triacylglycerol (mmol/L)	0.65±0.03[d]	3.22±0.21[a]	2.15±0.18[b]	1.09±0.04[c]
Urea (mmol/L)	17.47±1.55[b]	41.84±1.07[a]	36.38±2.29[a]	39.67±2.98[a]
Creatinine (μmol/L)	87.52±9.11[a]	101.66±4.95[a]	91.94±4.86[a]	84.87±2.03[a]
ALT (U/L)	24.04±4.59[a]	26.89±4.65[a]	27.33±4.63[a]	27.67±4.91[a]
AST (U/L)	26.61±4.59[a]	27.24±4.47[a]	24.71±4.16[a]	26.12±3.61[a]
IL-6 (pg/mL)	65.53±1.716 [b]	121.11±4.67[a]	116.31±4.853[a]	125.14±2.311[a]
TNF-α (pg/mL)	15.91±0.596[b]	22.09±1.299[a]	22.41±0.806[a]	24.52±0.563[a]
Liver L-MDA (mmol/g tissue)	0.008±0.001[b]	0.017±0.0003[a]	0.006±0.001[b]	0.019±0.001[a]
Hepatic glycogen (μg/mg tissue)	0.098±0.03[b]	0.094±0.02[b]	0.208±0.005[a]	0.157±0.005[a,b]

Note: Group and group II cited from El Barky et al (57).
Data are presented as (Mean ± S.E.).
Mean values with different superscript letters in the same column are significantly different at ($p \leq 0.05$).
[a, b, c, d, e] Indicated the difference or similarity between experimental groups. Control normal group (Group I), diabetic group (Group II), diabetic MSCs (Group III) and diabetic IPCs (Group IV).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; L-MDA, L-malonaldehyde; S.E., standard error; TNF- α, tumour necrosis factor- α.

Table 3
Serum and liver biochemical parameters in the different groups under study with treatment duration of 6 weeks

Parameter	Animal groups			
	I	II	III	IV
Glucose (mmol/L)	4.02±0.12[c]	21.83±1.25[a]	17.58±0.80[b]	21.44±0.55[a]
Insulin (pmol/L)	0.42±0.00[a]	0.07±0.00[d]	0.28±0.00[b]	0.14±0.00[c]
Glucose–insulin ratio	1232.24±87.82[d]	32334.67±1685.13[a]	7765.27±751.09[c]	19469.50±1058.52[b]
α -Amylase (U/L)	271.98±16.24[b]	539.49±44.02[a]	235.73±13.08[b]	250.056±16.78[b]
Adiponectin (pg/mL)	494.87±5.18[b]	1130.15±92.76[a]	556.58±44.83[b]	966.98±17.68[a]
Cholesterol (mmol/L)	1.87±0.04[a]	1.07±0.06[b]	1.14±0.07[b]	1.75±0.13[a]
Triacylglycerol (mmol/L)	0.67±0.01[a]	0.32±0.04[b]	0.57±0.05[a]	0.64±0.04[a]
Urea (mmol/L)	15.56±0.75[c]	27.16±1.40[b]	27.03±2.52[b]	33.42±1.43[a]
Creatinine (μmol/L)	950.0±6.54[b]	127.30±0.35[a]	94.59±5.75[b]	86.63±2.83[b]
ALT (U/L)	25.31±4.51[a]	24.51±3.23[a]	26.80±3.62[a]	23.13±3.62[a]
AST (U/L)	27.18±4.30[a]	25.75±2.54[a]	30.39±4.22[a]	26.82±4.28[a]
IL-6 (pg/mL)	69.46±0.374[b]	223.27±6.629[a]	83.9±4.960[b]	231.64±6.461[a]
TNF-α (pg/mL)	17.61±353[b]	46.60±3.493[a]	20.65±2.427[b]	45.49±2.653[a]
Liver L-MDA (mmol/g tissue)	0.004±0.0004[b]	0.02±0.005[a]	0.013±0.001[a]	0.015±0.002[a]
Hepatic glycogen (μg/mg tissue)	0.091±0.01[b]	0.078±0.01[b]	0.223±0.015[a]	0.170±0.03[a]

Note: Group I and group II cited from El Barky et al (57).
[a, b, c, d] Indicated the difference or similarity between experimental groups. Control normal group (Group I), diabetic group (Group II), diabetic MSCs (Group III) and diabetic IPCs (Group IV).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; L-MDA, L-malonaldehyde; TNF- α, tumour necrosis factor- α.

control group. Serum ALT and AST levels, which were detected in different treated groups, showed nonsignificant changes ($p>0.05$) in all experimental groups when compared with the control group (Tables 2 and 3).

Effect of cell transplantation treatment on proinflammatory markers

The measurements of the serum proinflammatory cytokines IL-6 and TNF-α revealed a significant increase ($p>0.05$) after 4 and 6 weeks in the STZ group when compared with the control group. Moreover, MSC-treated rats showed a nonsignificant change ($p>0.05$) in the values of both serum IL-6 and TNF-α after 4 weeks when compared with the STZ group and showed a significant increase ($p>0.05$) as compared with the control group. However, after 6 weeks, there was a significant decrease ($p>0.05$) of the values of IL-6 and TNF-α in the MSC-treated group as compared with the STZ group, and it reached the normal value as compared with the control group. The IPC-treated group had nonsignificant changes in both serum IL-6 and TNF-α levels after 4 and 6 weeks as compared with the STZ group and showed a significant increase as compared with the control group (Tables 2 and 3).

Effect of cell transplantation treatment on liver L-MDA and glycogen

The hepatic levels of L-MDA, a biomarker of lipid peroxidation, in the STZ group were significantly higher ($p<0.05$) than those in the control group after 4 and 6 weeks. In the MSC-treated group, the level of liver L-MDA was significantly reduced ($p<0.05$) after 4 weeks, and it reached the normal value as compared with the control group. Meanwhile, after 6 weeks, the change in the L-MDA level in the MSC-treated group was not significant as compared with the STZ group and the control group. However, in the IPC-treated group, the change in the value of L-MDA after 4 and 6 weeks was not significant as compared with those in the STZ group and the control group. Liver glycogen levels that have been detected in different treated groups showed a nonsignificant change ($p>0.05$) in the STZ group after 4 and 6 weeks as compared with the control group. Meanwhile, MSC-treated rats had a significant increase

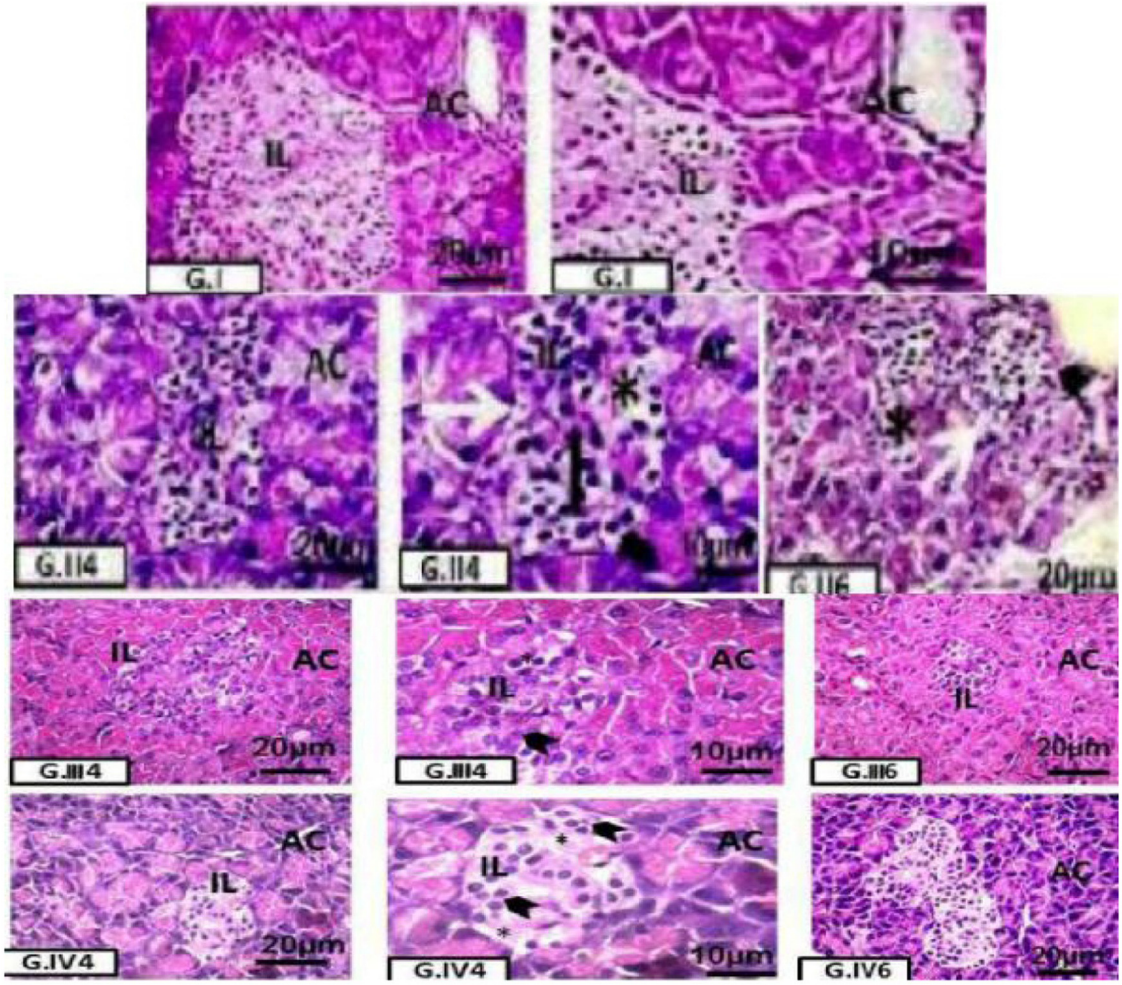

Figure 3. Photomicrograph of pancreatic tissue stained with hematoxylin and eosin. Group I (control rats) showed normal intact Langerhans islets (IL) embedded in the acinar cells (AC). Group II (diabetic rats after 4 weeks), which received streptozotocin, showing destructed Langerhans islets (IL) embedded in the acinar cells (AC), infiltration by inflammatory cells (black arrows), necrotic areas (asterisk), cells with vacuoles (white arrows) and cells with no cytoplasmic secretion (arrowhead). Group II (diabetic rats after 6 weeks) after receiving streptozotocin showing severe destructed Langerhans islets (IL) embedded in the acinar cells (AC). Note the increase of necrotic areas (asterisk), cells with vacuoles (white arrow) and cells with no cytoplasmic secretion (arrowhead). Group III (diabetic rats after receiving MSCs for 4 and 6 weeks, respectively: Langerhans islets (IL) had some apoptotic cells (asterisk) and an abundance of cells with vesicular nuclei (arrowhead). Group IV Group I and group II cited from El Barky et al (57). (diabetic rats after receiving IPCs for 4 and 6 weeks, respectively): Langerhans islets (IL) had an abundance of apoptotic cells (arrowheads) and necrotic areas (asterisk).
Note: Group I and group II cited from El Barky et al (57).

($p<0.05$) in liver glycogen after 4 and 6 weeks as compared with the rats in the STZ and control groups. Furthermore, the IPC-treated group had a nonsignificant increase after 4 weeks. This increase became significant ($p<0.05$) after 6 weeks when compared with the STZ group and control groups (Tables 2 and 3).

Histopathologic findings

Examination of the pancreas sections obtained from the control rats showed intact Langerhans islets with well-defined edges and plump islet cells. Examination of the pancreatic sections of the diabetic rats showed extensive destruction of Langerhans islets. The islets had ill-defined boundaries, and some necrotic areas were often noticed. Severe inflammatory cell infiltrations were observed. Some endocrine cells had vacuolization in their cytoplasm, and others had no cytoplasmic secretion. Examination of the pancreatic sections of the diabetic rats treated with MSCs showed that the islets still had ill boundaries. However, necrotic cells were rarely noticed within the islets. Langerhans islet cells appeared with vesicular eosinophilic stained cytoplasm. Examination of the pancreatic sections of the diabetic rats treated with IPCs showed an abundance of apoptotic cells within the islets. Moreover, necrotic areas were noticed (Figure 3).

Discussion

Islet transplantation has lately been shown to ameliorate hyperglycemia in diabetes, but the limited supply of human islet tissue and the use of long-term immune system repression to prevent allogeneic graft rejection prevent this therapy from being used in patients with type 1 diabetes (13). In the present study, BMSCs were isolated from adult male albino rats. At the end of the expansion phase, the cells became homogenous, spindle shaped and fibroblast-like and were arranged in monolayers (32). MSCs in culture were characterized by their adhesiveness and fusiform shape, which is in agreement with the findings of Abdel Aziz et al (33). MSCs retained their adherence in culture, while all of the other nonadherent cells were eliminated by washing during media change. Moreover, the selective medium and the polystyrene-coated tissue culture flasks enhanced the adhesion properties of the MSCs (34). The data

indicate that MSCs were CD^{-34} and CD^{+105}. Cultured MSCs are uniformly and strongly positive for CD105, CD90 and CD73, regardless of their passage or time in culture (35). Moreover, Gabr et al (32) state that flow cytometric analysis showed that MSCs expressed high levels of CD73, CD90 and CD105 but negligible levels of CD14, CD34 and CD45. These results indicate that the majority of the bone marrow–derived cells were MSCs. In addition, Li-Bo et al (36) found that MSCs could successfully differentiate in vitro into pancreatic islet beta-like cells. These cells are morphologically similar to pancreatic islet cells. MSCs can be differentiated into IPCs, and their therapeutic potential for diabetes depends on this differential ability (37). Moreover, Neshati et al (38) indicate that treatment of MSCs with high glucose, nicotinamide and 2-mercaptoethanol differentiates them into IPCs. These cells could be used as a model to develop stem cell–based therapies for DM (39).

The data from the present study show that the differentiated cells secreted around 15.01±1.04 nIU/mL insulin at a high glucose level. Insulin-secreting MSCs can be generated safely and effectively and demonstrate in vitro glucose-responsive alterations in insulin secretion levels (40).

DM is a global health disease; it is a metabolic disorder characterized by an increase in serum blood glucose levels (41). DM results from defects in insulin secretion, action or both. The chronic hyperglycemia can lead to diabetic complications, which are considered a major cause of morbidity and mortality (42). The chronic hyperglycemia resulting from diabetes can lead to irreversible damage, dysfunction and failure of various organs (41).

Insulin-dependent DM type 1 is a metabolic disease usually resulting from autoimmune-mediated beta-cell destruction requiring lifetime exogenous insulin replacement. MSCs hold promise as a viable novel therapeutic option for insulin-dependent DM (43).

Our morphologic observation, confirmed by determining the body weights of all experimental rats, showed that there was a significant reduction in body weight in the STZ group. STZ caused a significant reduction in body weight, which may be attributed to increased muscle wasting and lack of tissue proteins (44). The data obtained in the present study indicate that both the MSC-treated group and the IPC-treated group had a significant decrease in body weight after 4 weeks, which is in agreement with the findings of Wan et al (45) who reported that rats with diabetes did not improve their weight after STZ injection and after BMSC transplantation as compared with their body weight before STZ injection. However, the data obtained in the present study showed a significant increase in the body weight of MSC-treated rats and IPC-treated rats after 6 weeks, which is in agreement with the findings of Abdel Aziz et al (46), who state that the body weight of rats with STZ-induced diabetes has been improved by MSCs. This suggests that partial in vivo MSC differentiation resulted in a significant increase in serum fasting insulin, which led to improvement not only blood glucose levels but also in most metabolic parameters including body weight (47).

In this study, the STZ group showed an elevation in serum glucose levels and a decrease in serum insulin concentration as compared with the control group. STZ is a toxic substance that can induce selective destruction of pancreatic beta cells (48), which causes a significant increase in the serum glucose level, which might be a result of STZ causing a notable decrease in insulin release (49). In the present study, a significant decrease in serum glucose levels and a significant increase in serum insulin levels were observed in the MSC-treated group. MSCs are capable of lowering blood glucose levels in both animals and patients with diabetes (50). The mechanism of the therapeutic effect of MSCs on hyperglycemia might include islet restoration through direct differentiation into functional beta cells (51). Although a significant increase in serum insulin levels was observed in the IPC-treated group as compared with the STZ group, the glucose level could not be restored to the normal value, likely because the released insulin is not sufficient to decrease the blood glucose levels.

Our results showed that there was a significant increase in the serum glucose-to-insulin ratio in the STZ group. These results are in agreement with the findings by Ding et al (52) who observed a dramatic decrease in beta-cell function index and an increase in glucose-to-insulin ratio in rats with alloxan-induced diabetes compared with controls, which might be due to loss of beta-cell function and a severe decrease in insulin secretion and hyperglycemic state (53).

Conversely, in both the MSC and IPC treatment groups, the glucose-to-insulin ratio was ameliorated. MSCs can ameliorate hyperglycemia by modulating the insulin sensitivity of peripheral target tissues, possibly by upregulating glucose transporter type 4 expression and elevating phosphorylated insulin receptor substrate 1 and protein kinase B levels in insulin target tissues (54).

The results of the present study demonstrate a significant increase in serum α-amylase activity in the serum of the STZ group. The increment of serum α-amylase activity was modulated by both MSC and IPC treatment. BMSCs may enhance pancreatic reconstruction by activating the generation of tubular complexes (55). Also, Huang et al (56) state that MSCs reduced amylase and lipase activity in the serum of rats with damage to the pancreas and restored the necrotic pancreatic tissue. MSCs might also prevent inflammation and be involved in the reaction by producing some soluble materials.

The results also demonstrate that the serum adiponectin level was significantly increased in the STZ group, which is in accordance with findings by El Barky et al (57). Serum adiponectin levels were elevated in patients with type 1 DM and in patients with genetically defective insulin receptors who have microvascular complications when compared with healthy control subjects (4). Meanwhile, in both the MSC- and IPC-treated groups, adiponectin levels were significantly decreased. Adipose-derived MSCs represent a very promising approach to diabetes because they are endowed with a large number of bioactive mediators, such as leptin, adiponectin and visfastin, which are known to regulate glucose homeostasis (58).

Regarding the lipid profile, the present study showed a significant increase in serum total cholesterol and triacylglycerol concentrations after 4 weeks in the STZ group. The increment in total cholesterol level is attributed to the increased beta-oxidation of long-chain fatty acids and increased oxidation of ketogenic amino acids producing excess hepatic acetyl-coenzyme A, which is used for cholesterol synthesis (59,60). Adipose tissue–derived stem cell transplantation decreased the serum levels of total cholesterol and triacylglycerol, which may be due to MSCs improving hyperglycemia and thus increasing the insulin level, which is responsible for activation of lipoprotein lipase, which is responsible for removal of accumulated triacylglycerol (61). Significant decreases in both serum total cholesterol and triacylglycerol in the STZ group were observed after 6 weeks. The decrease in the lipid profile may be due to rats' need for more energy rather than glucose, so lipids were broken down to obtain energy, which is in agreement with findings of El Barky et al (57). Because treatment with both MSCs and IPCs ameliorates lipid profile levels, the decrease may be due to MSCs keeping the lipid profile within normal proportions.

Urea and creatinine levels were increased in the STZ group after 4 and 6 weeks, although after 6 weeks, the level of urea was decreased more than after 4 weeks, which may be because rats with diabetes were obtaining energy via gluconeogenesis, resulting in the breakdown of protein. DM is the major cause of renal morbidity and mortality. The metabolic abnormalities observed in uncontrolled diabetes result in gluconeogenesis (62). Human BMSCs were able to inhibit renal injuries and pancreatic islet degeneration (63). MSC treatment was deemed to be excellent in controlling hyperglycemia, improving the renal structure and function changes and reducing renal laminin expression. This improvement could be

related to the role of BMSC treatment in restoring the kidney architecture (64).

The present study showed significant increases in both serum TNF-α and IL-6 in the STZ group. TNF-α and IL-6 were produced by infiltrating macrophages, lymphocytes and monocytes, which damaged the pancreatic beta cells and produced type 1 DM by promoting the formation of oxygen free radicals, lipid peroxides and aldehydes (65). The MSC-treated group had significant reductions in both serum IL-6 and TNF-α. These results were an accordance with results of the study by Yoshimatsu et al (66) who reported that inflammation after transplantation of MSCs to mice with STZ-induced diabetes was significantly repressed by MSC co-transplantation. The level of the proinflammatory cytokine IL-6 and the magnitude of the immune reaction, as evidenced by bleeding and neutrophil infiltration at the early stage after transplantation, were significantly reduced by MSC co-transplantation. However, the mechanism of the anti-inflammatory effect of MSCs is not clear.

The present study showed that a significant increase was observed in the liver L-MDA concentration in the STZ group. High glucose levels can stimulate free radical production. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and nonenzymatic antioxidants (67). Conversely, the MSC-treated group had a significant decrease in the level of L-MDA, which is in agreement with findings by Fang et al (68) who reported that in rats with diabetes that were treated with autologous transplantation of adipose-derived MSCs, increases in malondialdehyde and carbonyl protein were significantly inhibited in comparison with the vehicle-treated and untreated diabetic animals.

In the present study, both the MSC- and IPC-treated groups showed a significant increase ($p<0.05$) in liver glycogen levels. The increase in liver glycogen may be attributed to increased glucose utilization in the liver rather than insulin secretion. The conversion of glucose into glycogen in the liver depends on the concentration of glucose and availability of insulin, which stimulates glycogen synthesis, which occurs in the presence of the enzymes glycogen synthase and glycogen phosphorylase. Synthase phosphatase activates glycogen synthase resulting in glycogenesis (69).

Conclusion

Stem cells, which can differentiate into IPCs, would provide a potentially free source of islet cells for transplantation and mitigate the major limitations of availability and allogeneic rejection. Therefore, the use of stem cells is becoming the most favourable therapy for DM. The treatment duration of 6 weeks is better than 4 weeks. Further study may result in changes to the protocol of stem cell therapy to improve diabetes treatment.

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