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ORIGINAL ARTICLE

A preliminary study of the use of human adipose tissue-derived stem cells for the treatment of streptozotocin-induced diabetes mellitus in a rat model

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Abstract The purpose of this study is to determine if intraventricular injection of human adipose tissue-derived stem cells (hADSC) are effective in treating streptozotocin (STZ)-induced diabetes mellitus (DM) in nude rats. Twenty-two adult, male nude rats (strain Crl:NIH-Fox1^{RNU}) were used to induce diabetes using streptozotocin. A single, 150 mg/kg STZ was injected intraperitoneally. Severity of the induced diabetic state was assessed by daily monitoring of body weight, clinical signs, and blood glucose levels. C-peptide was assessed before ADSC injection (T0) and at 3, 5, and 21 days after ADSC injection. Eight rats (40%) developed DM within 24 h after STZ injection. Of the eight rats that developed DM, five were given 2 million freshly prepared ADSC intraventricularly under echocardiography guidance 10 days after STZ injection and three were only given sterile saline for comparison. Surviving rats were humanly sacrificed 21 days after ADSC injection. The average weight of diabetic rats decreased significantly after STZ injection. ADSC injection had no effect on the body weight of rats. Non-fasting serum glucose levels increased significantly in both groups. In diabetic rats, C-peptide decreased significantly before ADSC injection and seemed to return to normal 21 days after ADSC administration. Results of this preliminary study might suggest a beneficial effect of using hADSC for the treatment of STZ-induced diabetes in adult nude rats.

Keywords Streptozotocin · Nude rats · Diabetes · Adipose-derived stem cells

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Introduction

Diabetes mellitus is a chronic, widely spread human disease. Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to induce diabetes mellitus in laboratory animals with variable success and many difficulties. Surgical removal of the pancreas is an effective method; however, to induce diabetes, at least 90–95% of the pancreas has to be removed (Akbarzadeh et al. 2007). Injection of anterior hypothesis extract has been used to induce diabetes with less reliable results (Rastellini et al. 1997). Another method which is more uniformly effective and widely used is the injection of streptozotocin.

Streptozotocin (STZ; *N*-nitro derivative of glucosamine) is a naturally occurring, broad-spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic, insulin-producing beta cells in mammals (Hayashi et al. 2006; Szkudelski 2001; Takeshita et al. 2006; Weiss 1982). Induction of experimental diabetes in the rat using streptozotocin is very convenient and simple to use (Brosky and Logothetopoulos 1969).

Clinically, symptoms of diabetes are clearly seen in rats within 2–4 days following single intravenous or intraperitoneal injection of 60 mg/kg STZ (Elias et al. 1994). Alternatively, much effort has been made to use the renewable source of stem cells. The possibility that human bone marrow stem cells (hBMSC) may be useful in enhancing insulin secretion and that multipotent stromal cells from human bone marrow can provide a potential therapy for human diabetes mellitus have been investigated previously (Lee et al. 2006; Voltarelli et al. 2007; Zuk et al. 2001). In this preliminary study, we test the hypothesis that human ADSC injected inside the left ventricle for treatment of DM is helpful in alleviating signs of diabetes mellitus in nude rats.

Materials and methods

Animals

Twenty-two adult, male nude rats (average body weight 275±25 g; strain Crl:NIH-Fox1^{RNU}, Charles River, Wilmington, MA, USA) were used to induce diabetes. Animals were housed individually in special clear-sided cages at controlled temperature (22°C) with a 12:12 h light:dark cycle and had free access to water and chow diet over a 2-week adaptation period. Body weights were measured twice weekly. All experimental procedures were approved and supervised by the Jordan University of Science and Technology Animal Care and Use Committee which conforms to the principles laid by the National Research Council Guide for the Care and Use of Laboratory Animals.

Induction of diabetes

Rats were fasted for 12 h before diabetes was induced using STZ. Rats received a single intraperitoneal (i.p.) injection of 150 mg/kg of STZ (Sigma, St. Louis, MO, USA). STZ was freshly dissolved in 0.05 M citrate buffer, pH 4.5. For the i.p. injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone–iodine solution, and the designated amount of STZ was injected in the caudal abdominal cavity using sterile 25 gauge needle. Following induction of diabetes, rats were monitored daily for abnormal clinical signs and body weight changes.

Blood glucose levels

Severity of the induced diabetic state was assessed by daily monitoring of blood glucose levels. For the determination of blood glucose using Glucocheck (Biotest Medical Corp., Tortola, VI, USA), whole blood was obtained from the tail vein from all rats immediately before STZ injection and daily until euthanatized. Animals whose blood glucose level exceeded 200 mg/dl at 24 h after treatment were considered diabetic. Rats with blood glucose levels exceeding 400 mg/dl were administered 10 U insulin (Mixtrad 30, Novo Nordisk, Denmark) subcutaneously.

Stem cell harvest and isolation

Adipose-derived stem cells used for injection in this study were obtained from human patients who underwent abdominal wall liposuction at the University of Jordan Hospital. Fat was washed three times using PBS buffer (Gibco, USA). The fat was digested by adipase (Sigma) at 37° for 40 min with shaking followed by centrifugation for 5 min at 700×*g*. The supernatant was removed and the cell pellet was aspirated and transferred into a new tube for washing. Washed cells were placed in suspension media and centrifuged again for 5 min at 700×*g*. Then the pellet was transferred into a new conical tube for a second wash. The pellet was then transferred into a new conical tube and suspended in 10-ml suspension media and aspirated into a 20-ml delivery syringe. The suspension containing the cells was filtered through 200-μm filters. Cells in the filtered solution were counted and re-suspended to obtain a final concentration of 10 million/ml. Cells were stained with

Table 1 Average body weight, serum glucose levels, and C-peptide concentrations in STZ-induced diabetic rats with ADSCs treatment (group I) and without ADSCs treatment (group II)

Parameter	Group							
	Group 1 (N=5)				Group II (N=3)			
	T0	Day 3	Day 5	Day 21	T0	Day3	Day 5	Day 21
Weight (g)	270±21	260±27	246±28*	245±33*	283±28	267±26*	246±30*	254±29*
Glucose (mg/dl)	129±33	238±54*	269±20*	281±19*	138±34	373±45*	279±36*	257±18*
C-peptide (ng/ml)	0.39±0.19	0.25±0.12*	0.33±0.17	0.36±0.50	0.45±0.22	0.21±0.13*	0.20±0.11*	0.17±0.09*

*P value ≤0.05

DAPI and DiL stains and transferred chilled for transplantation within 2 h.

Cell injection technique

Rats were randomly allocated into two groups and received either 0.2 ml of sterile saline (control group I; n=3) or group II received 2 million hADSCs (0.2 ml) intraventricularly 10 days after STZ injection (n=5). Whole blood was collected in plain blood tubes at T0 and on days 3, 5, 21 (non-fasting) following hADSC injections for the determination of serum glucose levels and C-peptide. Serum was separated using a centrifuge (ALC Centrifugette 4206, Milano, Italy) at 1,500×g for 5 min. Serum was then placed in labeled plastic tubes and stored at −20°C until testing. Glucose serum levels were determined using Trinder method (Glucose GOP-PAP). Serum C-peptide concentration was determined using radioimmunoassay.

Statistical analysis

Results are expressed in mean ± SD. Data were analyzed using one-way analysis of variance and Duncan's multiple range test or non-parametric statistics. Statistical analyses were performed using Graphpad Prism for Windows (Graphpad, San Diego, CA, USA). P value ≤0.05 was considered significant.

Results and discussion

Average body weight, serum glucose levels, and C-peptide concentrations in diabetic rats before and after hADSC administration are illustrated in Table 1. Out of 20 nude rats who received STZ, only eight (40%) developed diabetes based on non-fasting glucose measurements. These results are comparable with our previous findings of STZ-induced diabetes in nude rats (Abu Abeeleh et al. 2009). The average weight of diabetic rats decreased significantly after STZ injection. As expected, the body weight of diabetic rats decreased significantly over the observation period. Similarly, the blood glucose increased significantly from 129±33 mg/dl before STZ injection to 238±54 mg/dl 3 days after STZ injection. In diabetic rats, serum C-peptide levels decreased significantly indicating insufficient insulin production by the pancreatic beta cells due to STZ.

Diabetes mellitus in its two common forms, type 1 and type 2, are the result of inadequate mass of functioning β cells. To function properly, diabetic patients must restore pancreatic islets by regeneration of β cell islets. There are several therapeutic strategies for diabetes mellitus including oral medication, insulin injection, and transplantation of pancreas or islets with variable success. More recently, more effective methods to cure diabetes, the use of stem cells, have been considered. It was found that bone marrow (BM)-derived cells are capable of transdifferentiation into pancreatic β cells (Ianus et al. 2003). However, other studies have not found evidence of such transdifferentiation although the capacity of BM-derived stem cells to initiate pancreatic differentiation has been described (Choi et al. 2003; Lechner et al. 2004; Taneera et al. 2006; Hess et al. 2003). In order to investigate the potential role of hADSCs in the regeneration of pancreatic islets using the rat model, we injected 2 million hADSCs in STZ-induced diabetic rats. Although we found no significant effect of ADSCs injection on the body weight of rats, C-peptide decreased significantly before ADSCs injection and seemed to return to normal levels 21 days after ADSCs administration, indicating possible role for the injected ADSCs in the regeneration of β cells and the return of its function. Although these results are encouraging, further investigation of the mechanisms and effectiveness of hADSCs in the treatment of diabetes is warranted.

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