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Ameliorative effects of bone marrow derived pancreatic progenitor cells on hyperglycemia and oxidative stress in diabetic rats

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ABSTRACT

The present study aimed to investigate the effects of Bone marrow derived pancreatic progenitor cells (BM-PPCs) in diabetic rats. It was conducted on 30 adult male Sprague-Dawley rats weighing 200–220 g. They were divided into three groups: (a) Group 1 was the control group; (b) Group 2 was the diabetic (induced diabetic by a single intraperitoneal (IP) injection of streptozotocin (STZ) (60 mg/kg) and (c) Group 3 was the treated (received injection of 2.5 X 10^6 BM- PPCs via the tail vein twice with a 21-day time interval). The blood glucose level was estimated weekly, the oxidative stress and insulin gene expression were evaluated at the end of the experiment. Pancreatic tissue histopathology was performed. The insulin immuno-histochemical reaction was applied to the islets. The blood glucose level was reduced in the treated group over time till reaching its acceptable level whereas it was increased in the diabetic group. The oxidative stress was decreased in the treated group compared to the diabetic one. The treated group showed increased expression of the insulin gene compared to the diabetic group. The immune-histochemical analysis of insulin showed an increased number and size of pancreatic islets in the treated group compared to the diabetic one. Thus, the twofold injection of BM- PPCs could restore the normal beta-cell morphology and function.

1. Introduction

Diabetes is one of the top ten leading causes of morbidity and mortality, affecting nearly 350 million people worldwide (Xi and Bu, 2014). The International Diabetes Federation has reported that more than 300 million people had diabetes in 2011 and that this number is assumed to be expanded to 552 million by the year 2030. It has been also reported that diabetes caused 4.6 million deaths in 2011 (Whiting et al., 2011).

The pathogenesis of diabetes mellitus (DM) is closely related to persistent hyperglycemia and glucose autoxidation which is reflected into various oxidative activities including reactive oxygen species (ROS) production; causing cellular damage and oxidative stress in both animals and humans (Wolff et al., 1991; Davi et al., 2005; Kowluru and Chan, 2007). The paraoxonase (PON) gene family consists of three members: PON1, PON2 and PON3 (La Du and Kulow, 1992; Primo-Parmo et al., 1996). The PON2 is ubiquitously expressed in humans and appears to play a protective role against oxidative stress (Witte et al., 2012; Ng et al., 2005, 2006; Reddy et al., 2008). Previous studies have shown that PON2 may be associated with several diseases such as vascular disease, leukemia and diabetes (Mackness et al., 2005; Pan et al., 2002; Sanchez et al., 2006).

Streptozotocin (STZ) induces diabetes in rats and demonstrates significant defects in antioxidant defense elements. In addition, it increases ROS generation which is one of the major determinants of diabetic complications (Kakkar et al., 1995; Opara, 2002). Therefore, researchers have used it to create an experimental model of diabetes as it is a simple, economic and available substance (Akbarzadeh et al., 2007, Skurikhin et al., 2017).

Different strategies of diabetes treatment have been used. Oral and injected hypoglycemic therapy and surgical tissue transplantation have been used with different degrees of succession. Replacement therapy with stem cells has become a novel method for the treatment of type 1 diabetes mellitus (T1DM). Mesenchymal stem cells (MSCs) derived from bone marrow (BM), fat, umbilical cord and cord blood can be induced to be differentiated into cells with insulin secretion function (Cara et al., 2017; Ho et al., 2012; Kim et al., 2012).

Thus, the present study attempted to examine the innovative

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potential role of BM- PPCs for the treatment of T1DM.

2. Materials and methods

2.1. Animal model and groups

For achieving the purpose of the present study, 30 adult male Sprague-Dawley rats (weighing 200–220 g) were used. They were purchased from Faculty of Medicine, Cairo University, Egypt. They were maintained under standard conditions of temperature (25 ± 2 °C) and natural light-dark cycle. All animals had free access to drinking water and rat pellet diet. The ethical guidelines for animal handling were supervised by the animal facilities, Faculty of Medicine, Cairo University which was in compliance with the standards of the National Guide for Care and Use of Laboratory Animals.

The rats were allowed 7 days of acclimation prior to the experimental treatment. They were divided into two main groups. Group 1 (G1) (n = 10) received a single injection of a 1ml saline solution (IP) and it was kept as the control group. The second main group (n = 20) was injected with a single dose of streptozotocin (60 mg/kg, IP) dissolved in 0.01 M citrate buffer, pH 4.5, immediately before use. Three days later, blood glucose levels were determined in whole blood samples collected from the tail vein. The rats injected with STZ were considered diabetic if the fasting blood glucose levels were 250 mg/dl or higher (Akbarzadeh et al., 2007; Yavuz et al., 2003). Diabetic animals were further divided into two groups of 10 rats each. Group 2 (G2), was kept as the diabetic group; Group 3 (G3), termed the treated group, was diabetic and injected with BM- PPCs ($2.5X10^6$ cells, IV) twice with a 21-day time interval (Fig. 1).

2.2. Induction of diabetes

G2 (the diabetic group) and G3 (the stem cells-treated group) received single intraperitoneal injection of STZ (60 mg/kg) which was purchased from Sigma-Aldrich (St. Louis, MO, USA). The blood glucose levels were measured after 3 days to make sure that the levels exceeded 250 mg/dl (Akbarzadeh et al., 2007).

2.3. BM-MSCs

Stem cells were obtained from the Biochemistry Department, Faculty of Medicine, Kasr Al-Aini, Cairo University. They were provided as first-passage culture cells suspended in phosphate buffered saline (PBS).

2.4. Preparation of BM-MSCs

Bone marrow was harvested by flushing the tibiae and femora of six-week-old female Sprague-Dawley rats with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Alhadlaq and Mao, 2004). Nucleated cells were isolated and suspended in complete culture medium supplemented with 1% penicillin-streptomycin. The cells were incubated at 37 °C in 5% humidified CO_2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80-90% confluence), the culture was washed twice with PBS and the cells were trypsinized with 0.25% trypsin in 1 mm EDTA for 5 min at 37 °C. After centrifugation, the cells were suspended with serum-supplemented media and incubated in the culture flask. The resulting culture was referred to as the first-passage culture. MSCs in culture were characterized by their adhesiveness and fusiform shape under the phase-contrast microscope (Rochefort et al., 2005).

2.5. Identification of BM- PPCs by flow cytometry

The harvested Bone marrow were incubated with microbeads-conjugated antibodies characteristic for BM-MSCs for 30 min at 4 °C. Then analyzed with magnetic assistant cell sorter (MACS) technique using the MiniMACS system microBead Kit (Miltenyi Biotec, Bergish Gladbach, Germany) according to the manufacturer's instructions. Antibodies specific for MSCs characterization, CD34+, CD45+, CD73+, CD90+ and CD105+, were used. The cells were washed and then proceed to magnetic separation and the labeled cell fraction CD105, CD73, and CD90 was taken as markers for pancreatic progenitor cells (Zou et al., 2017; Lee et al., 2016; Zanini et al., 2011).

2.6. MSCs transplantation

The animals of treated group were anesthetized by injection of a mixture of ketamine hydrochloride (100 mg/kg, IP) and xylazine (5 mg/kg, IP). 2.5×10^6 cells/rat of BM- PPCs in 1.0 ml saline was injected via the tail vein. After 21 days, the second injection of BM-PPCs was performed (Fig. 1).

2.7. Assessment of in vivo migration of BM- PPCs

BM- PPCs were labeled by fluorescence cell membrane tracker. CM-DiI labeling of mesenchymal stem cells has been used to track their distribution in vivo and differentiation following transplantation. 1 ml of CM-DiI was added to cells after the removal of culture medium. CellTracker CM-DiI is a DiI derivative that is water-soluble, thus facilitating the preparation of staining solutions for cell suspensions and fixed cells. In addition to its improved solubility in culture medium, CellTracker CM-DiI contains a thiol-reactive chloromethyl moiety that allows the dye to covalently bind to cellular thiols. Thus, the label is well retained in some cells throughout fixation and permeabilization steps. Membrane staining with CellTracker CM-DiI persists following routine paraffin processing. The cells were incubated at 37 °C for 5 min. and then in 4 °C for 15 min. Next, the cells were incubated overnight after the addition of the culture medium. After 24 h, about 1×10^6 BM-PPCs were injected in the tail vein of the treated group. After 24 h of stem cell injection, one animal was anesthetized and serial sections from the pancreas were prepared after paraffin impregnation. The pancreatic tissues were examined under the fluorescent microscope where the slides were stained by Hoechst staining solution (Sigma-Aldrich, St. Louis, MO, USA).

The validity of this system was established by staining of pancreatic tissue of normal rat injected by BM- PPCs previously incubated with phosphate buffered saline instead of CM-Dil (negative control) (Fig. 5a).

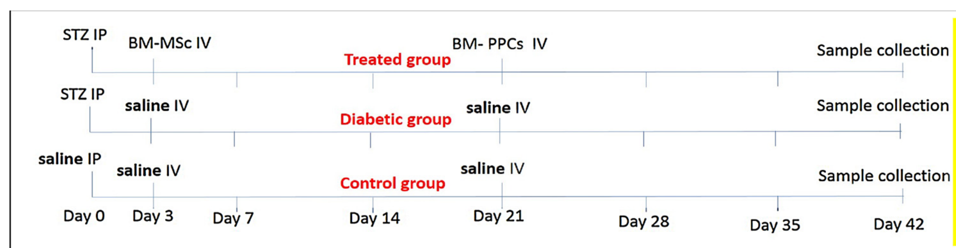

Fig. 1. Diagram showing the timeline of the treatment procedure.

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