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Original Research

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

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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.

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.
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.