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Effect of human umbilical cord blood-derived mononuclear cells on diabetic nephropathy in rats

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ABSTRACT

Diabetic nephropathy (DN) is damage to the kidney which can lead to chronic renal failure, eventually requiring dialysis. Diabetes mellitus is the most common cause of adult kidney failure worldwide in the developed world. The current work was designed to elucidate the effect of mononuclear cells (MNCs) injection on reverse DN in rats exposed to streptozotocin (STZ) injection compared to metformin as a known hypoglycemic drug, 40 Male rats were divided equally into 4 groups; normal control group, diabetic control group, MNCs group were diabetic rats treated with MNCs (30×10^6 MNCs/rat once iv dose) in the tail vein of the rat, and metformin group were diabetic rats treated with metformin (100 mg/kg orally daily dose) for four weeks. The results indicated an improvement effect of MNCs and metformin on STZ-induced DN in rats, which was evidenced by significant decrease in urinary albumin/creatinine ratio, N-acetyl-β-D-glucosaminidase (NAG), urinary kidney injury molecule-1 (KIM-1), serum urea, serum creatinine and fasting blood glucose and significant increase in C- peptide level, compared to diabetic control group. Additionally MNCs treated group exhibited pronounced effects in all previous parameters compared to metformin treated group. It is proved that MNCs treatment was superior to metformin in controlling hyperglycemia, and improving renal function in diabetic rats.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or action [1]. One of the most important complications of this metabolic disease is diabetic nephropathy (DN), which is now considered the principal cause of end-stage renal disease (ESRD) [2].

Traditional diabetes therapy divided into two main groups; insulin preparations and oral hypoglycemic drugs. Insulin preparations have a disadvantage of allergic reactions, hypoglycemia, weight gain and edema. Oral hypoglycemic drugs have several side effects like hypoglycemia, weight gain, nausea, edema, anemia, abdominal discomfort, diarrhea, flatulence, Lactic acidosis, pulmonary edema, hepatic and congestive heart failure [3]. Additionally, traditional therapy has a higher cost on long term usage as diabetic patient using the drug therapy all his life.

Cell therapies with human embryonic and adult stem cells have emerged as an alternative management for various diseases [4]. These cells were able to proliferate and differentiate into various cell types including those bearing a phenotype of insulin-secreting β-cells [5]. Additionally, stem cells have the ability to lower renal injury, accelerate tubular proliferation and improve kidney function [6].

The umbilical cord is the transport of nutrients and oxygen between mother and fetus. The term human umbilical cord blood (HUCB) describes blood remaining in the cord after birth [5]. HUCB-MNCs is a source of stem cells, which has several advantages over other stem cell sources. It is easy procurement, no risk to donors, low risk of transmitting infections, easy, availability, non-invasive, low risk of graft-versus- host diseases (GVHD) and lack of related ethical issues [7]. HUCB is an attractive source of stem cells for various diseases such as brain ischemia therapy [8], bladder dysfunction [9], myocardial infarction [10], and renal dysfunction [11].

The current study was designed to elucidate the role of MNCs in improving the renal function changes associated with streptozotocin (STZ)-induced diabetic nephropathy in rats, as well as controlling diabetes, and proliferation of insulin secreting β-cells. Metformin was used as a reference drug.

2. Materials and methods

2.1. Experimental design

Male albino rats were used in the study, 170–200 g each. They were purchased from Faculty of Veterinary medicine, Zagazeag University,

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Egypt. They were weighed and housed in cages for 2 weeks under identical environmental conditions and allowed free access to food and water ad libitum. The care and handling of animals were approved in accordance with the guidelines for the care and use of laboratory animals approved by Research Ethics Committee (Faculty of Pharmacy, Tanta University, Egypt).

For induction of diabetes, STZ was obtained from Sigma-Aldrich Chemical Company, USA, dissolved in disodium citrate buffer (pH 4.5) and injected i.p to rats at a dose of 55 mg/kg [12]. 72 h later, blood was collected from tail vein after overnight fasting for 12 h, and serum samples were analyzed for blood glucose. Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for further investigations [13].

Diabetic rats were randomly divided into 3 groups; diabetic control group, MNCs group, and metformin group. A fourth group of normal rats served as the normal control. MNCs (30×10^6 MNCs/rat) were injected once in the tail vein of the rat [14]. Metformin (Enzo Life Sciences Inc., USA) was given to rats by oral gastric tube at a dose of 100 mg/kg [15] daily for four weeks. Normal control and diabetic control rats received the vehicle.

Rats were fasted overnight and 24 h urine samples were collected. Blood samples were utilized for separation of serum, and urine was centrifuged for 15 min at 3000 rpm for measurement of the renal markers. Blood and urine samples were collected on day 5, 14 and 28 after start of experiment.

2.2. MNCs separation

Human umbilical cord blood (HUCB) was collected in the presence of EDTA disodium salt as anticoagulant agent, then diluted 1:1 in PBS (Biochrom AG, Berlin). Eight mL of diluted cord blood was transferred onto 4 mL ficoll (Biochrom AG, Berlin) and was centrifuged 20 min at 3000 rpm at room temperature. The interface which contains the low density MNCs was collected and suspended in equal volume of PBS and mixed gently, then centrifuged 10 min at 1500 rpm, 4 °C (Eppendorf AG, Germany). The pellet was taken, resuspended in PBS and centrifuged 10 min at 1500 rpm and maintained at 2–8 °C until injection within 24 h from blood collection [16].

Counting of MNCs was done using homocytometer (Improved Neubauer, China) and calculated according to the equation:

Mean × Dilution factor × 10^4 × Number of milliliter of cells [17].

The viability of MNCs was examined by trypan blue dye 0.4% exclusion staining (Sigma-Aldrich, USA) and calculated as:

% Viability = Number of viable cells/Number of total cells × 100 [17].

2.3. Determination of fasting blood glucose

The concentration of fasting blood glucose was determined enzymatically according to [18] using glucose oxidase (GOD) to catalyze the oxidation of glucose to hydrogen peroxide and gluconic acid, using kits obtained from BioMed (Germany).

2.4. Determination of serum C- peptide

Level of C- peptide in serum was measured using rat C- peptide ELISA kit obtained from SunRed Biotechnology company (Shanghai, China), according to manufacturer protocol.

2.5. Determination of serum urea

The serum urea level was determined spectrophotometry according to [19]using kits obtained from Biodiagnostics (Cairo, Egypt), based on urease- Berthelot method, which yield a blue dye indophenol product absorbing light at 540 nm.

2.6. Determination of creatinine

Serum and urinary creatinine concentration were determined colorimetrically according to [20] using kits obtained from Biodiagnostics (Cairo, Egypt), according to manufacturer instruction. Picric acid reacts with creatinine in alkaline solution to form a colored complex which was measured at 495 nm using spectrophotometer (Unico 2100, Dayton, NJ), and the concentration was expressed as mg/dL.

2.7. Determination of urinary albumin

The urinary albumin concentration was measured by using microalbumin kit strips obtained from Bayer Healthcare LLC (USA), according to manufacturer protocol, and urinary albumin was expressed as albumin/creatinine ratio.

2.8. Determination of urinary N-acetyl-β-D-glucosaminidase (NAG) activity

NAG activity was determined with sodium 3-cresolsulfonphthaleinyl-*N*-acetyl-β-D-glucosaminide according to [21] using kits obtained from Roche (Germany), according to manufacturer instruction. The color produced was measured colorimetrically at 580 nm, the NAG activity was presented as U/L.

2.9. Determination of urinary KIM-1

Level of KIM-1 in urine was measured using rat TIM-1/KIM-1 ELISA kit obtained from R&D Systems, Inc. (Canada, USA), according to manufacturer protocol.

3. Statistical analysis

Analysis of data was performed with statistical package for social science version 20 (SPSS software). Data are presented as mean ± SD. Statistical comparison between groups and within groups was performed by one-way analysis of variance (ANOVA). Statistical comparison intra group was performed by paired student t- test. Statistical significance was obtained at $P > 0.05$.

4. Results

4.1. Effect on fasting blood glucose

Fasting blood glucose showed a significant elevation ($p < 0.05$) in the STZ group (526.25 ± 65.35 and 515.88 ± 67.05 mg/dL) compared to the normal control group (109.38 ± 12.01 and 113.88 ± 11.56 mg/dL) after 5 and 14 days respectively from STZ injection. On day 14, MNCs and metformin treated groups exhibited a significant decrease in fasting blood glucose level ($p < 0.05$) compared to diabetic control group (37.3% and 12.3%, respectively). However the percent of reduction was maximum on day 28 of the experiment (72.1% and 57.4%, respectively) (Table 1).

4.2. Effect on C- peptide level

As shown in Table 2, diabetic control group showed a significant decrease in C-peptide level compared to normal control group ($p < 0.05$, 74.7%). On day 28 of the experiment, treatment with either MNCs or metformin exhibited a significant increase of C-peptide level (3.3 fold and 1.8 fold, respectively, $p < 0.05$) compared to diabetic control group. MNCs group also showed significant ($p < 0.05$) increase in C-peptide level compared to metformin treated group (2.1