



Mesenchymal Stem Cells Ameliorate Lipid Metabolism while Reducing Blood Glucose in Streptozotocin (STZ)-Induced Diabetes Mellitus and High Fat Diet Induced Obesity

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Abstract

Background: Mesenchymal stem cells (MSCs) are among the most frequently used cell type for regenerative medicine and have beneficial effects to treat different pathologies, including neurological disorders, cardiac ischemia, diabetes, and bone and cartilage diseases. Fortunately, MSCs, known for their lower immunogenicity and self-renewal ability, can be induced into insulin-producing cells (IPCs) and have attracted significant attention for the treatment of DM. Importantly.

Material & methods: This study was done in a period from July 2022 to May 2023. The study involved a local strain of Ninety adult healthy male albino rats weighing 100- 150 g, were purchased from animal house in Faculty of Medicine of Zagazig University. Rats were kept in steel wire cages (50x30x20 cm), under hygienic conditions, 4-5 rats per cage, All rats were kept at a comfortable temperature (20 to 24 C), had free access to water and maintained on normal light-dark cycle. Before starting the study, the animals were acclimated to these conditions for 2 weeks. **Results:** There were significant decreases in serum levels of MDA in Streptozotocin (STZ)-induced diabetic HUCBMSCs treated. However, this effect was insignificant in Streptozotocin (STZ)-induced diabetic combined insulin and HUCBMSCs treated group when compared with Streptozotocin (STZ)-induced diabetic HUCBMSCs treated group.

Conclusion: Cord blood derived Mesenchymal stem cells infusion into diabetic rats are able to home to injured pancreatic tissue, differentiate into β -cells and improve the metabolic parameters in both types of diabetes.

Key words: Mesenchymal stem cells, lipid metabolism, blood glucose ,streptozotocin (STZ), diabetes mellitus,high fat diet , obesity.

Introduction

Diabetes mellitus (DM) is a chronic endocrine disorder characterized by improper glucose, lipid, and protein metabolism. Diabetes is defined by a decrease in the circulating levels of insulin (insulin deficit) and a decline in the responsiveness of tissues to insulin (insulin

insensitivity) [1]. Type 1 diabetes mellitus (T1DM) is quite prevalent in the world, with a proportion of 1 in every 300 persons and steadily rising frequency of incidence of about 3% every year. The incidence of T1DM among infants is also increasing, with children as

young as 6 months succumbing to it, instead of that at a rather established vulnerable age of around seven and near puberty, when the hormones antagonize the action of insulin^[2, 3].

Diabetes rates have risen dramatically in Middle Eastern countries over the last 2 decades. Impaired glucose tolerance (IGT) affects 48 million adults in the International Diabetes Federation (IDF) Middle East/North African (MENA) region, predisposing them type 2 diabetes mellitus (T2DM)^[4].

T2DM is a heterogeneous disease. It was recently proposed that diabetic patients can be stratified into different subgroups with differing disease progression and risk of diabetic complications^[5, 6].

An alternate way of treating diabetes mellitus currently consider stem cell-based therapies. Mesenchymal stem cells (MSCs)^[7], which are known for their potential to differentiate into multiple cell lineages, are now considered an ideal tool for developing new cell-based therapeutic approaches

Adult MSCs can be extracted from various sources, including the umbilical cord blood, placental tissue, adipose tissue, peripheral blood, lung, liver, heart, bone marrow (BM), testes, pancreas, spleen, and dental pulp. Among the different types of MSCs, bone marrow-derived MSCs (BM-MSCs) and adipose-derived MSCs (AMSCs) are the most frequently used cells^[8, 9]

Moreover, it was found that umbilical cord MSCs were similar to MSCs in the bone marrow and could be induced to differentiate into adipogenic cells, osteogenic cells, cardiomyogenic cells, and insulin-producing cells are now considered an ideal tool for developing new cell-based therapeutic approaches^[10].

Mesenchymal stem cells (MSCs) (<https://doi.org/10.1155/2021/9938658>) are among the most frequently used cell type for regenerative medicine and have beneficial effects to treat different pathologies, including

neurological disorders, cardiac ischemia, diabetes, and bone and cartilage diseases^[11]. Fortunately, MSCs, known for their lower immunogenicity and self-renewal ability, can be induced into insulin-producing cells (IPCs) and have attracted significant attention for the treatment of DM. Importantly^[12].

The aim of this study is to test the ability of umbilical cord derived MSCs in regaining of β -cells structure and function and to study its effect on insulin resistance in experimentally induced type I and II diabetes mellitus in rats.

Material and Methods

Animals

This study was done in a period from July 2022 to May 2023. The study involved a local strain of Ninety adult healthy male albino rats weighing 100- 150 g, were purchased from animal house in Faculty of Medicine of Zagazig University.

Rats were kept in steel wire cages (50x30x20 cm), under hygienic conditions, 4-5 rats per cage, All rats were kept at a comfortable temperature (20 to 24 C), had free access to water and maintained on normal light-dark cycle^[13]. Before starting the study, the animals were acclimated to these conditions for 2 weeks^[14].

Isolation and Expansion of Mesenchymal stem cell from cord blood

Cord blood was collected from the umbilical cord vein with informed consent of the mother on sodium citrate buffer. In a sterile Petri dish, the umbilical cord was cut into small pieces of about 1.0 mm. The tissue fragments were washed with PBS and centrifuged at 1900 r/min for 6 min. The tissue fragments were suspended with 25 mL of serum-free stem cell culture medium, inoculated into a T175 culture flask, and placed in an incubator at 37 °C and 5% CO₂ concentration. The primary cells were harvested when the confluence reached 80%. When the adherent cells were

passed to the fourth generation, after 72 h of cell culture, the supernatant was collected as UC-MSC-conditioned medium (UC-MSC-CM) and centrifuged at 500 g for 10 min and passed through a 0.22- μ m filter (Millipore) before use. The fifth generations of cells were used in this animal experiment. Human UC-MSC induction differentiation experiments were carried out using Oil red O staining to confirm adipogenesis, using Alizarin red staining to verify osteogenesis, and Alcian blue staining to examine chondrogenesis. Related cell surface markers were detected by flow cytometry^[16]. This method was done with collaboration with clinical pathology department at faculty of medicine, Zagazig University. We used the first generation of MSCs detached by 0.25% trypsin then centrifuged at 2000 RPM for 5 min and then washed two times with PBS

Experimental Design and diabetes induction

Ninety adult healthy male albino rats were divided into equal groups each group contains 10 animals. These groups are: Group (I) control group: normal fed diet, which received 3.41 kcal/g of normal chow diet for 12 weeks. Calories were distributed as follows: 6% fat, 17% proteins, and 77% carbohydrates^[16]. Group (II) Streptozotocin (STZ)-induced diabetes group (n = 40 rats) subdivided into: untreated, Insulin treated, HUCBMSCs treated and combined insulin and HUCBMSCs treated,^[17] Group (III) high-fat-diet-induced obesity group: received 5.6 kcal/g of a HFD for 12 weeks. Calories were distributed as follows: 58% fat, 18% proteins, and 24% carbohydrates^[18]. The food iron content was the same for both food groups (n = 40 rats) subdivided into untreated, insulin treated, HUCBMSCs treated and combined insulin and HUCBMSCs treated. In insulin treated groups (Humulin 70/30) by subcutaneous injection twice daily, in a dose designed according to blood glucose level that is measured before injection. In Streptozotocin (STZ)-induced diabetes groups, intraperitoneal injection of freshly prepared

solution of streptozotocin at a dose of 60 mg/kg dissolved in saline due to instability of STZ in aqueous media^[17].

In HUCBMSCs treated groups, human male umbilical cord blood-derived MSCs (5x10⁶ cells/200 μ l) were injected IV once in tail vein, Animals from each group were sacrificed on day 14 after CB-MSCs infusion^[19]. A 25 mg/kg intraperitoneal dose of sodium thiopental was used to anaesthetize rats. At the conclusion of the study, blood samples from the retro-orbital plexus were taken. Blood samples were obtained by exsanguination at the time of killing, collected and allowed to clot for 2 h at room temperature before centrifugation. Sera were stored at -20°C until analysis.

Laboratory analysis

For all animals, the following parameters were measured: fasting serum insulin level^[20], fasting serum glucose level^[21], Calculation of homeostasis model assessment (HOMA) and β -cell function (HOMA- β). [HOMA-IR = insulin (μ U/mL) x glucose (mmol/L) / 22.5] and β -cell function [HOMA- β = 20 x insulin (μ U/mL) / (glucose (mmol/L) - 3.5)]^[22], Estimation of serum total cholesterol levels^[21], serum high – density lipoprotein (HDL)-cholesterol^[23], serum triglycerides^[24]. Determination of low-density lipoprotein cholesterol^[25], Measurement of serum malondialdehyde (MDA)^[26], Streptozotocin, [N-(Methylnitrosocarbonyl)- α -D-glucosamine kits^[17]

Diabetes induction was confirmed by criteria of diabetes such as weight loss, glucosuria and hyperglycemia by measurement of blood glucose level in each animal (blood sampled from the tail vein) with the Bionime GM300 Glucometer, animals were considered diabetic if their FBG level was >250 mg/dL^[27].

Statistical analysis

Results were presented as mean \pm SD. Statistical analysis was performed using the statistical package for the social sciences,

version 19.0 (SPSS; SPSS Inc. Chicago, Illinois, USA). Repeated measures of analysis of variance was applied followed by least significance differences for multiple comparisons. Levels of significance (P) were considered statistically significant when P value was less than 0.05 [28]

Results

Impact of HUCBMSCs administration on streptozotocin (STZ)-induced diabetic rats

There were significant decreases in serum levels of glucose in Streptozotocin (STZ)-induced diabetic HUCBMSCs treated. However, this effect was insignificant in Streptozotocin (STZ)-induced diabetic combined insulin and HUCBMSCs treated

group when compared with Streptozotocin (STZ)-induced diabetic HUCBMSCs treated group. Likewise, there were significant decreases in HOMA IR in Streptozotocin (STZ)-induced diabetic HUCBMSCs treated group. In addition, there were significant decreases in total cholesterol, LDL and triglycerides, together with a significant increase in HDL in Streptozotocin (STZ)-induced diabetic HUCBMSCs treated. There were significant decreases in serum levels of MDA in Streptozotocin (STZ)-induced diabetic HUCBMSCs treated. However, this effect was insignificant in Streptozotocin (STZ)-induced diabetic combined insulin and HUCBMSCs treated group when compared with Streptozotocin (STZ)-induced diabetic HUCBMSCs treated group (Table 1).

Table 1: Serum changes, HOMA and oxidative stress measures in control and Streptozotocin (STZ)-induced diabetes groups.

		Control (I)	Streptozotocin (STZ)-induced diabetes group untreated (IIa)	Streptozotocin (STZ)-induced diabetes group insulin treated (IIb)	Streptozotocin (STZ)-induced diabetes group HUCBMSCs treated (IIc)	Streptozotocin (STZ)-induced diabetes group insulin and HUCBMSCs treated (IId)
Glucose (mmol/L)	$\bar{X} \pm SD$	4.31±0.387	6.8±0.649	5.99±0.71	4.95±0.57	4.73±0.368
		P< 0.001** ^(a)		P< 0.05* ^(b)	P< 0.001** ^(c)	P=0.3 ^(d)
Insulin (µIU/ml)	$\bar{X} \pm SD$	9.6±1.43	15.79±1.48	11.1±0.99	9.7±1.04	8.2±0.79
		P< 0.001** ^(a)		P<0.001** ^(b)	P< 0.001** ^(c)	P< 0.001** ^(d)
HOMA IR	$\bar{X} \pm SD$	1.84±0.338	4.56±0.63	2.58±0.40	1.08±0.34	1.61±0.20
		P< 0.001** ^(a)		P< 0.001** ^(b)	P< 0.001** ^(c)	P< 0.001** ^(d)
HOMA β	$\bar{X} \pm SD$	41.24±6.9	45.49±7.28	29.37±5.56	29.84±3.39	32.7±4.38
		P= 0.1 ^(a)		P< 0.05* ^(b)	P< 0.001** ^(c)	P=0.12 ^(d)
Total cholesterol (mg/dl)	$\bar{X} \pm SD$	174.8±11.21	276.1±17.14	196.5±8.73	183.8±7.86	182±6.13
		P< 0.001** ^(a)		P< 0.001** ^(b)	P< 0.001** ^(c)	P=0.57 ^(d)
Triglycerides (mg/dl)	$\bar{X} \pm SD$	68.3±13.12	96.2±9.53	122.9±4.4	110.2±6.19	86.7±5.77
		P< 0.001** ^(a)		P< 0.001** ^(b)	P< 0.001** ^(c)	P< 0.001** ^(d)
LDL (mg/dl)	$\bar{X} \pm SD$	65.1±14.17	138.1±9.55	96.1±4.5	88.1±4.6	85.6±4.30
		P< 0.001** ^(a)		P< 0.001** ^(b)	P< 0.001** ^(c)	P=0.23 ^(d)
HDL (mg/dl)	$\bar{X} \pm SD$	64.6±6.15	37.5±5.5	44.8±4.75	59.1±6.11	60.6±5.13
		P< 0.001** ^(a)		P< 0.05* ^(b)	P< 0.001** ^(c)	P=0.73 ^(d)
MDA (mmol/l)	$\bar{X} \pm SD$	8.13±0.033	18.42±0.43	10.52±0.72	9.9±0.61	9.43±0.57
		P> 0.001** ^(a)		P> 0.001** ^(b)	P< 0.05* ^(c)	P=0.09 ^(d)

n=10 in each group. Data are represented as mean± SD. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride,(MDA); Malondialdehyde; * ,Significant; ** highly significant. **(a)** P value between group (I) and group (IIa), **(b)** P value between group (IIa) and group (IIb), **(c)** P value between group (IIb) and group (IIc), **(d)** P value between group (IIc) and group (IId).

Impact of HUCBMSCs administration on High fat diet (HFD) induced diabetic rats:

There were significant decreases in serum levels of glucose in High fat diet induced diabetic obese HUCBMSCs treated groups.

Likewise, there were significant decreases in HOMA IR in High fat diet induced diabetic obese HUCBMSCs treated group. In addition, there were significant decreases in total cholesterol, LDL and triglycerides, together with a significant increase in HDL in High fat diet induced diabetic obese HUCBMSCs treated group. In high fat diet induced diabetic Combined insulin and HUCBMSCs treated group, there was significant increases in HOMA B when compared with High fat diet induced diabetic HUCBMSCs treated group. In addition, there were significant decreases in serum levels of MDA in High fat diet induced diabetic obese HUCBMSCs treated group **(Table 2)**.

Table 2: Serum changes, HOMA and oxidative stress measures in control and high fat diet studied groups:

		Control (I)	High fat diet induced diabetic obese group untreated (IIa)	High fat diet induced diabetic obese group Insulin treated (IIb)	High fat diet induced diabetic obese group HUCBMSCs treated (IIc)	High fat diet induced diabetic obese group insulin and HUCBMSCs treated (IId)
Glucose (mmol/L)	$\bar{X} \pm SD$	4.22±0.32 P< 0.001** ^(a)	6.8±0.54 P<0.05* ^(b)	6.14±0.63	4.98±0.59 P< 0.001** ^(c)	4.61±0.47 P< 0.05 * ^(d)
Insulin (µIU/ml)	$\bar{X} \pm SD$	9.6±1.17 P< 0.001** ^(a)	16.43±1.84	11.4±1.07 P< 0.001* ^(b)	9±1.33 P< 0.001** ^(c)	8.9±1.37 P=0.8 ^(d)
HOMA IR	$\bar{X} \pm SD$	1.8±0.24 P< 0.001** ^(a)	4.9±0.71	3.11±0.35 P< 0.001** ^(b)	1.99±0.50 P< 0.001** ^(c)	1.82±0.37 P=0.39 ^(d)
HOMA β	$\bar{X} \pm SD$	42.21±6.48 P=0.22 ^(a)	45.40±4.66	34.18±6.73 P< 0.001** ^(b)	32.67±3.54 P> 0.05 ^(c)	38.53±3.73 P< 0.05* ^(d)
Total cholesterol (mg/dl)	$\bar{X} \pm SD$	179±10.26 P> 0.001** ^(a)	277.6±17.43	239.6±14.48 P< 0.001** ^(b)	186.4±8.64 P< 0.001** ^(c)	179.6±6.42 P< 0.05* ^(d)
Triglycerides (mg/dl)	$\bar{X} \pm SD$	68.7±13.10 P> 0.001** ^(a)	123.6±4.58	108.2±8.20 P> 0.0001** ^(b)	86.3±5.67 P< 0.0001** ^(c)	77.8±4.94 P< 0.05* ^(d)
LDL (mg/dl)	$\bar{X} \pm SD$	64.9±14.08 P> 0.0001** ^(a)	138.2±8.39	105.9±5.7 P> 0.001** ^(b)	96.4±4.65 P< 0.001** ^(c)	77.5±25.48 P< 0.05* ^(d)
HDL (mg/dl)	$\bar{X} \pm SD$	66.3±5.27 P< 0.001** ^(a)	37.7±5.58	44.8±4.85 P< 0.05* ^(b)	55.1±5.42 P< 0.001** ^(c)	60.6±5.13 P< 0.05* ^(d)
MDA (mmol/l)	$\bar{X} \pm SD$	8.17±0.038 P> 0.001** ^(a)	18.49±0.44	11.52±1.66 P< 0.001** ^(b)	9.96±0.58 P< 0.05* ^(c)	9.32±0.67 P< 0.05* ^(d)

n=10 in each group. Data are represented as mean± SD. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride,(MDA); Malondialdehyde; * ,Significant; ** highly significant. **(a)** P value between group (I) and group (IIa), **(b)** P value between group (IIa) and group (IIb), **(c)** P value between group (IIb) and group (IIc), **(d)** P value between group (IIc) and group (IId).

Discussion

Diabetes mellitus, a disorder of metabolism, results in the elevation of glucose level in the blood. In this hyperglycemic condition and many complications [29]. High-fat diets are a likely cause of low-grade inflammation and obesity-related pathologies (progression of T2DM, insulin resistance and glucose intolerance) [30].

Our results we found glycemic control, improvement of lipid profile and decrease of inflammatory markers after MSCs treatment in agreement with MSCs holds great promise for treating pathophysiology, as well as tissue engineering and regeneration for treatment of diseases, such as in diabetes mellitus [31]. In addition, Stem cell therapy [PMID: 32746936] [15], has become the most likely new breakthrough in the treatment of DN due to its self-renewal capacity, multilineage differentiation potential, paracrine effects, and immunomodulatory properties. Mesenchymal stem cells (MSCs) are a class of adult stem cells derived from mesoderm that have strong self-renewal and multi-directional differentiation potential.

We used umbilical cord mesenchymal stem cells in our study in agreement with The umbilical cord has been proved to be an easy-access, reliable, and useful source of mesenchymal stem cells (MSC) for clinical applications due to its primitive, immunomodulatory, non-immunogenic, secretory and paracrine, migratory, proliferative, and multi-potent properties [32].

The present study showed significantly decreased fasting serum glucose level and serum insulin level in both stem cell-treated diabetic groups after cord blood derived mesenchymal stem cells (CB-MSCs) infusion in agree with **El-Sawah SG et al** [33], who reported that the therapeutic benefits of MSCs in alleviating metabolic anomalies and hepato-renal diabetic complications.

This is in line with **Xiang E SG et al** [15], who found that UC-MSCs can effectively improve the renal function, inhibit inflammation and fibrosis, and prevent its progression in a model of diabetes-induced chronic renal injury, indicating that UC-MSCs could be a promising treatment strategy for diabetic nephropathy.

Moreover, these results are in agreement with **Jana et al** [34], who suggested that the blood glucose level decreased significantly in all treatment groups (DM rats plus MSCs into the head and tail of the pancreas and whole pancreas) 14 d after the administration of MSCs and prevented additional increases in blood glucose in the treated diabetic rats.

In our results, we found glycemic control, improvement of lipid profile and decrease of inflammatory markers in agreement with others who reported that the increased lipid fractions levels were reverted back to near normal values as a consequence to MSCs injection compared to the diabetic untreated rats [32].

In addition, after tail vein injection, hucMSC effectively reduced blood glucose, maintained body weight and improved renal function in diabetic kidney disease rats [35, 36].

Consequently, **Fernando et al** [37], proved that MSC administration not only increased the number of insulin-producing cells, but also restricted glucagon producing cell expansion.

The molecular mechanism by which MSCs participate in the treatment of diabetes remains unclear. The possible mechanisms include promoting islet cell regeneration, reducing insulin resistance in peripheral tissues, increasing insulin sensitivity, regulating the immune system, protecting islet β -cells, and improving diabetic complications and Stem cells were suggested to exert a therapeutic effect mainly by replacing damaged tissues [38, 39].

In contrast, **Yiling et al** [40], proved that the beneficial effect of a single infusion of MSCs

in ameliorating hyperglycemia in diabetic rats, was maintained only for a period not exceeding 4 weeks. He suggested that the limited number of MSC-derived functional β -cells in vivo and the small amount of insulin produced by these cells.

Consistent with our data, **Antony et al**^[41], found dramatically lipoprotein disturbance in STZ diabetic rats; as a result of the significant serum TL, TG, TC, LDL-C and VLDL-C levels increase with HDL-C decrease. Once accumulated in tissues, lipids can generate species that will lead to increased inflammation and oxidative stress inducing the progression of many diabetic complications.

Moreover, MSCs administration to diabetic animals significantly improved the lipid profile as shown by the significant decrease in TGs, TC, LDL-C, while a significant increase in HDL-C, compared to the diabetic untreated mice^[42].

In addition, streptozotocin (STZ)-induced type 2 diabetic rats on a high-fat diet (40% of calories as fat) for 2 weeks and showed that endurance training improved dyslipidemia in HFD/STZ rats. Dyslipidemia, which is associated with T2DM ^[43].

HucMSC-ex partially reversed insulin resistance in T2DM indirectly to accelerate glucose metabolism, increased storage of glycogen in the liver to maintain glucose homeostasis and HucMSC-ex inhibited STZ-induced β -cell apoptosis ^[44]. Moreover, MSCs substantially suppressed damage of β cells and enhanced their repair as indicated by the improvement of glucose, insulin, insulin resistance, and β cell function ^[45]. Treatment with a single dose of MSCs has greatly improved insulin sensitivity in diabetic developing male rats ^[46].

Our study indicated that hucMSCs has antidiabetic and anti-inflammatory properties as Intravenous injection of hucMSCs could prevent renal fibrosis and proteinuria by

inhibiting inflammation and could protect human glomerular mesangial cells by reducing oxidative damage and apoptosis ^[47, 48], indicating anti-diabetic potential of hucMSCs.

In our study infused MSCs for 14 days so limitation to our study infused MSCs predominantly accumulated in pancreatic tissues at 28 days post infusion. The effects of MSCs on preserving β -cell function and modulating inflammation tended to be dose-dependent ^[49].

In agreement with treatment of the diabetic developing rats with MSCs during the early phase evoked significant alleviation in all the measured oxidative stress indices (the levels of MDA, TAS, TOS, and OS) ^[50]

The current study showed that improvement of diabetic condition after single dose infusion of undifferentiated cord blood-derived MSCs in both diabetic groups (T1D & T2D). In agreement with the discovery of mesenchymal stem cells and their unique immunomodulatory and multipotent properties has inspired therapies to treat diabetes by essentially reversing the conditions causing the disease and has different approaches to treat both types of diabetes ^[51, 52, 53].

Conclusion

Cord blood derived Mesenchymal stem cells infusion into diabetic rats are able to home to injured pancreatic tissue, differentiate into β -cells and improve the metabolic parameters in both types of diabetes.

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- through inhibiting inflammation and fibrosis. *Stem Cell Res Ther.* 2020 Aug 3; 11(1):336. doi: 10.1186/s13287-020-01852-y. PMID: 32746936; PMCID: PMC7397631.
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