Effect of Mesenchymal Stem Cells (M.S.C.) in Streptozotocin (STZ) induced type I diabetic rats.

Sameh Elsonbaty[1]; and Ashraf kotb[2]

Histology[1]; and Physiology[2] Departments, Faculty of Medicine, October 6 University, Egypt.
drashrafsalem@hotmail.com

Abstract: Diabetes is one of the most important causes of mortality and morbidity all over the world. Renewal of functional pancreatic islets has been a goal of stem cell biologists since early 2000. Since that time, many studies have reported successful creation of glucose-responsive pancreatic beta-cells. **Aim of work:** This work aimed to study the effect of MSC on Streptozotocin (STZ)- induced diabetes in male albino rats to detect its potential therapeutic effect and its possible application to humans. **Material and methods:** Thirty male albino rats (150 – 170 grams) were included in this study. They were divided into three equal groups: Group I (control), group II (diabetic), and group III (diabetic group treated with MSC). Diabetes was induced by intraperitoneal injection of STZ (60 mg/kg). MSC were injected intravenously into the rat tail vein in the group III and left for two months Glucose and Insulin levels were measured for the three groups at the beginning of the study and after two months. Diabetic group (group II) showed significant higher glucose levels while there was a significant lower insulin levels compared to control group. Group III showed higher insulin and lower glucose level compared to group II. **Conclusion:** treatment with MSCs. showed significant lower levels of glucose and higher levels of insulin compared to diabetic group.

[Sameh Elsonbaty; Ashraf kotb. **Effect of Mesenchymal Stem Cells (MSC) in Streptozotocin (STZ) induced type I diabetic rats.** *J Am Sci* 2013;9(9):276-280]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 36

Key Words: STZ (Streptozotocin), MSC (Mesenchymal stem cells).

1.Introduction

Diabetes is actually a group of diseases characterized by abnormally high levels of the sugar glucose in the bloodstream. This excess glucose is responsible for most of the complications of diabetes, which include blindness, kidney failure, heart disease, stroke, neuropathy, and amputations **(Chen et al; 2004).** Type 1 diabetes, also known as juvenile-onset diabetes, typically affects children and young adults. Diabetes develops when the body's immune system sees its own cells as foreign and attacks and destroys them. As a result, the islet cells of the pancreas, which normally produce insulin, are destroyed. In the absence of insulin, glucose cannot enter the cell and glucose accumulates in the blood **(Beati *et al.*, 1997**).

There is currently no cure for diabetes. People with type 1 diabetes must take insulin several times a day and test their blood glucose concentration three to four times a day throughout their entire lives. Frequent monitoring is important because patients who keep their blood glucose concentrations as close to normal as possible can significantly reduce many of the complications of diabetes, such as retinopathy (a disease of the small blood vessels of the eye which can lead to blindness) and heart disease, that tend to develop over time**(Bonner *et al.*, 2000).**

Over the past several years, doctors have attempted to cure diabetes by injecting patients with pancreatic islet cells—the cells of the pancreas that secrete insulin and other hormones. However, the requirement for steroid immunosuppressant therapy to prevent rejection of the cells increases the metabolic demand on insulin-producing cells and eventually they may exhaust their capacity to produce insulin. The deleterious effect of steroids is greater for islet cell transplants than for whole-organ transplants (**Itken *et al.*, 2001).**

All tissues per se have the capacity of homeostasis maintenance. However, after injury this process is somehow disturbed by inflammation or by extracellular matrix disruption, not allowing proper stem cell action. Thus, in a way to assist the repair process several works have focused on the administration of exogenous stem cells **(Gabby *et al.*, 2006).**Researches have turned their attention to adult stem cell that appear to be precursors to islet cells and embryonic stem cells that produce insulin **(Assady *et al.*, 2001).** (Surprisingly, the administration of adult stem cell in several experimental diseases has showed to improve its clinical outcome and more over amelioration of tissue architecture. The mechanism of action that leads to this improvement is not well defined, whether it is due to fusion of dying cells with MSC, differentiation of MSC to other cell types or through a paracrine action, where several bioactive factors contribute to the main mechanism of action suggested by **Zuk *et al.*, in (2002**). Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells, are self-renewing cells

that can be found in almost all postnatal organs and tissues (**Porada *et al.*, 2006**). MSCs are most frequently isolated from bone marrow but can generally be derived from any organ **(Da silva *et al.*, 2006**). Depending on their intended purpose, experimental or therapeutic use, MSCs can be isolated from adipose tissue, umbilical cord blood, compact bone, and other tissues (**Ettla *et al.*, 2004).** The main functional characteristics of MSCs are their immune-modulatory ability, for self-renewal, and differentiation into tissues of mesodermal origin **(Addi *et al.*, 2008).** There is a possible therapeutic effect of MSCs in diabetes suggested by their capacity to generate insulin-producing cells(IPCs) (**Nautta and Febbe;2007).**These IPCs express multiple genes related to the development or function of pancreatic beta cells, including high expression of insulin (**Volarevic *et al.*, 2010**)] and were able to release insulin in a glucose-dependent manner that led to amelioration of diabetic conditions in streptozotocin (STZ)-treated rats (**Xie *et al.*, 2009**). Several lines of evidence suggest that in vivo hyperglycemia is an important factor for bone marrow-derived MSCs differentiation into IPCs capable of normalizing hyperglycemia in diabetic rats, including those with chronic hyperglycemia (**Tang *et al.*, 2004).**

2. Material and methods

Thirty white male albino rats aged one month (150-170 grams) were included in the present study. They were obtained from Ophthalmology Institute and were housed in wire mesh cages at room temperature and maintained on normal chow and had free access to water.

The rats were divided into three groups, each group ten rats

Group 1: Control group.

Group 2: Diabetic group using Streptozotosin (S.T.Z.).

Group 3: Diabetic group treated with MSC

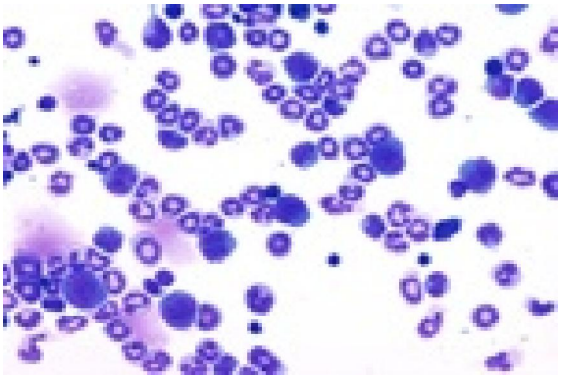
Diabetes was induced by injection of STZ at a dose of 60 mg./Kg/day (Fujita ***et al.,*** 2005)**.** M.S.C. were injected once intravenously in the rat tail.

Blood samples were taken at the beginning and at the end of experimental period for determination of glucose level and insulin level.

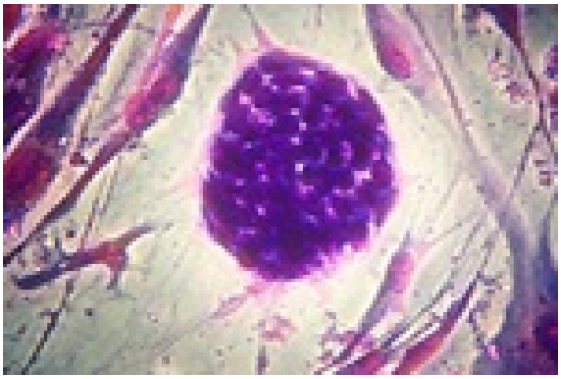
The glucose and insulin levels were measured by spectrophotometry and E.L.I.S.A. methods respectively.

* Preparation of BM-derived MSC

Rat diabetic models were made to test *in vivo* function of differentiated MSCs. Rat MSCs were isolated from bone marrow of Wistar rats nd cultured. Passaged MSCs were induced to differentiate into islet-like cells under following conditions: pre-induction with L-DMEM including 10 mmol/L nicotinamide + 1 mmol/L beta-mercaptoethanol+200 mL/L fetal calf serum (FSC) for 24 hrs, followed by induction with serum free H-DMEM solution including 10 mmol/L nicotinamide+1 mmol/L, beta-mercaptoethanol for 10 hrs. Differentiated cells were observed under inverse microscopy, and identified by expressing a gene called **PDX-1**, which encodes a protein that initiates transcription from the insulin gene. These genes, called cell markers, are useful in identifying particular cell types. insulin and nestin expressed in differentiated cells were detected with immunocytochemistry. Insulin excreted from differentiated cells was tested with radioimmunoassay.



Bone marrow aspirate



Isolated stem cells

Statistical Analysis

Statistics were done using SPSS methods.

3. Results

Table (1) showed serum levels for glucose and insulin in all studied groups.

Table (1): Insulin & Glucose levels for the control, diabetic, and the Diabetic M.S.C.-treated groups (Mean S±D).

	Control	Diabetic	Diabetic +M.S.C.
Insulin level IU/L	446.93 ± 102	50.9 ± 22	398.10 ± 76
Glucose level mg/dl	109.5 ± 3.2	243.2 ± 2.5	111.2 ± 3

* Significant (*P* value < 0.05).

Groups II subjected to STZ showed significant higher levels for glucose and lower levels for insulin when compared with the control group (*P* < 0.05).

Group III which received M.S.C. as a treatment showed a significant improvement in the levels of glucose and insulin in comparison to the levels without treatment (*P* < 0.05).

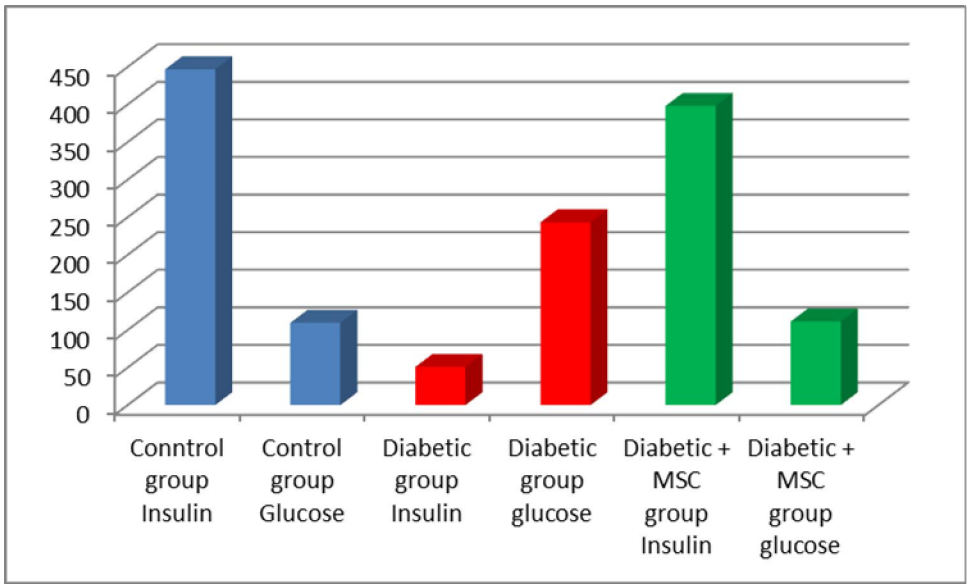


Figure (1): The insulin level (IU/L) and Glucose level (mg/dl) for the control group, the diabetic group and diabetic groups treated with stem cell(Mean S±D)

4. Discussion

Type 1 diabetes mellitus (T1D) is caused by an immune mediated destruction of the insulin producing β cells in the pancreas. β Cell destruction is irreversible and despite intensive insulin therapy the condition is connected with development of late diabetic complications and increased mortality (***Borch and Jhonson, 1989).***

In developing a potential therapy for patients with diabetes, researchers hope to develop a system that meets several criteria. Ideally, stem cells were able to multiply in culture and reproduce themselves exactly. That is, the cells should be self-renewing. Stem cells should also be able to differentiate *in vivo* to produce the desired kind of cells For diabetes therapy, it was not clear whether it will be desirable to produce only beta cells—the islet cells that manufacture insulin—or whether other types of pancreatic islet cells are also necessary. Studies by (**Soria *et al.*, 2000**), indicated that isolated beta cells—those cultured in the absence of the other types of islet cells—are less responsive to changes in glucose concentration than intact islet clusters made up of all islet cell types. Islet cell clusters typically respond to higher-than-normal concentrations of glucose by releasing insulin in two phases: a quick release of high concentrations of insulin and a slower release of lower concentrations of insulin. In this manner the beta cells can fine-tune their response to glucose. As multipolar nerve cells known as ganglion cells are responsible for activation of beta cells to secrete insulin (**Schuldiner *et al.*, 2000).**

The objective of the present study was to study the potential therapeutic effect of M.S.C. injection in STZ induced diabetes in rats. Rats were divided into 3 equal groups,1st one was the control group,2nd group received Steptozotocin to induce diabetes and the 3rd one received both Steptozotocin

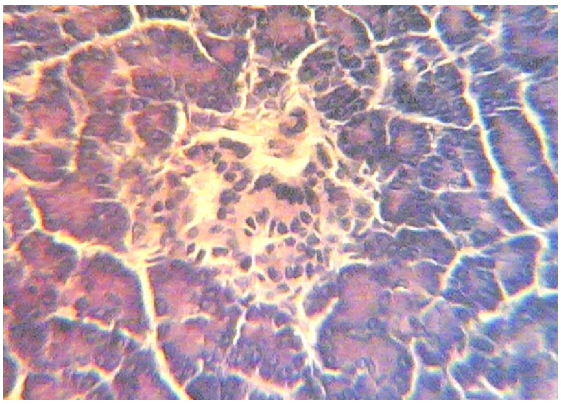
and MSCs. Results show a significant higher levels of glucose in group 2 (diabetic group) compared to control group (P >0.005), while the insulin level showed significant lower level in the diabetic group (P < 0.005), when compared with the same group. Group III which was treated with the MSCs. showed significant lower level of glucose in compared to the diabetic group with significant higher level of insulin in comparison to diabetic group. Improvement of insulin secretion may be due to MSCs. These results agreed with the results of **(Rottoli *et al.*, 2004).** They stated that the stem cells migrate to the site of damage and undergo differentiation and promote structural and functional repair and help to cure diabetes and thus restore the normal insulin level in the body.

Histological results:

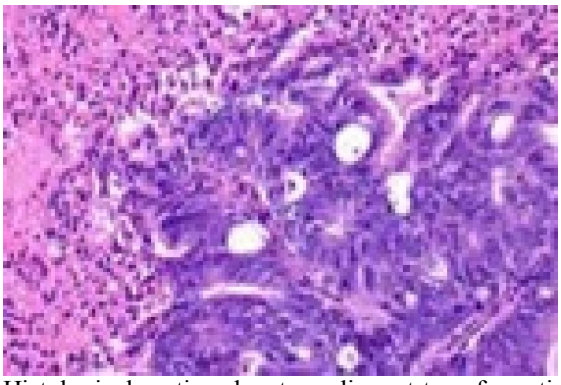
New islets of Langerhans reappear again after stem cell therapy in 7 rats.

No islets of Langerhans were detected in 2 of the rats.

It is important to denote that one of the rats who received stem cells showed malignant transformation of pancreas.



Functional areas of Islets of Langerhans appear again in pancreatic tail after stem cell therapy.



Histological section denote malignant transformation of pancreas of one rat

The results of the present study agree with **(Chen *et al.*, 2004)** whom stated that the Islet-like functional cells differentiated from marrow mesenchymal stem cells, may be a new procedure for clinical diabetes stem -cell therapy, as they can control blood glucose level in the diabetic rats, by islet differentiation.

The major problem in dealing with these cells is maintaining the delicate balance between growth and differentiation. Cells that proliferate well do not produce insulin efficiently, and those that do produce insulin do not proliferate well. According to the researchers, the major issue is developing the technology to be able to grow large numbers of these cells that will reproduce and produce normal amounts of insulin **(Levine; 2001).**

The discovery of the methods to isolate and grow human embryonic stem cells renewed the hopes of doctors, researchers, and diabetes patients and their families that a cure for type 1 diabetes, and perhaps type 2 diabetes as well, may be within striking distance. In theory, embryonic stem cells could be cultivated and coaxed into developing into the insulin-producing islet cells of the pancreas. With a ready supply of cultured stem cells at hand, the theory is that a line of embryonic stem cells could be grown up as needed for anyone requiring a transplant.

It is important to denote that one of the rats who receives stem cells show malignant transformation of pancreas so we cannot say that stem cell therapy iscompletely safe in humans.

References

1. Addi R, Fiorina P, Adra CN *et al.* Immunomodulation by Mesenchymal antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells 2004;10:3016–3020. 255–263.491.
2. Assady, S., Maor, G., Amit, M., Itskovitz-Eldor, J., Skorecki, K.L., and Tzukerman, M. (2001). Insulin production by human embryonic stem cells 53:1721–1732.
3. Beattie, G.M., Otonkoski, T., Lopez, A.D., and Hayek, A. (1997). Functional beta-cell mass after transplantation of human fetal pancreatic cells: differentiation or proliferation? Diabetes. 46, 244–248.
4. Bonner-Weir, S., Taneja, M., Weir, G.C., Tatarkiewicz, K., Song, K.H., Sharma, A., and O'Neil, J.J. (2000). *In vitro* cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. U. S. A. *97,* 7999–8004.
5. Borch and Johnsen K.*The prognosis of insulin-dependent diabetes mellitus.An epidemiological approach. Dan Med Bull1989;**36**:336–48*

6. Chen LB, Jiang XB, Yang L. Differentiation of rat marrow mesenchymal Clinical science Scientific reports; Inhibition of experimental diabetic cataract by topical administration of RS-verapamil hydrochloride: Br J Ophthalmol 2004; **88**:44-47
7. Da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006;119(part 11):2204–2213.
8. EttlA., DaxerA., GöttingerW., SchmidE. Gabbay JS, Heller JB, Mitchell SA, Zuk PA, Spoon DB, Wasson KL, *et al.* Osteogenic potentiation of human adipose-derived stem cells in a 3-dimensional matrix. Ann Plast Surg. 2006 Jul;57(1):89-93.
9. Fujita H.,Jin J.,Davis L. : Characterization of susceptibility of inbred mouse strains to diabetic nephropathy. Diabetes 54:2628-2637, 2005.
10. Itkin-Ansari, P., Demeterco, C., Bossie, S., Dufayet de la Tour, D., Beattie, G.M., Movassat, J., Mally, M.I., Hayek, A., and Levine, F. (2001). PDX-1 and cell-cell contact act in synergy to promote d-cell development in a human pancreatic endocrine precursor cell line. Mol. Endocrinol. *14,* 814–822.
11. Levine, F. cell-cell contact act in synergy to promote d-cell development in a human pancreatic endocrine precursor cell line.2001 Mol. Endocrinol. *14,* 814–822.
12. Nauta AJ, FibbeW.Immunomodulatory properties of mesenchymal insulin-producing cells obtained from murine bone marrow. Differentiation 2009;77:483.
13. Porada CD, Zanjani ED, Almeida-Porad G. Adult mesenchymal stem cells: A pluripotent population with multiple applications Curr Stem Cell Res Ther 2006;1:365–369.
14. Prockop DJ.Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71–74.
15. Schuldiner, M., Yanuka, O., Itskovitz-Eldor, J., Melton, D., and Benvenisty, N. (2000). Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A. *97,* 11307–11312.
16. Soria, B., Roche, E., Berná, G., Leon-Quinto, T., Reig, J.A., and Martin, F. (2000). Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin induced diabetic mice. Diabetes. *49,* 157–162.
17. Tang D., Cao L., Burkhardt B. Stem cells into pancreatic islet beta-cells. World J Gastroenterol stem cells: A potential therapeutic strategy for type 1 diabetes. stromal cells. Blood 2007;110:3499–3506.
18. Volarevic V., Al-Qahtani A.,Arsenijevic N,. Interleukin-1 receptor. Diabetes 2008; 57: 1759–1767.
19. Xie QP, Huang H, Xu B *et al.* Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002 Dec;13(12):4279-95.

8/23/2013