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شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

A Comparative Histological Study on the Role of Umbilical Cord Mesenchymal Stem Cells Versus Their Conditioned Medium on the Pancreatic Beta Cells in Experimentally Induced Diabetes Mellitus in Albino Rat

Histological study
Thesis
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بسم الله الرحمن الرحيم

صدق الله العظيم

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Dedication

To:

My parents

for their endless love, support, and continuous care

My Husband & My Family

- ***To my sons, to my friends***
- ***To my professors***

Thank you all

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List of Abbreviations

Abb.:		*Full term*
AD-MSCs	:	Adipose derived mesenchymal stem cells
ADSC-CM	:	Adipose derived stem cells- conditioned medium
BDNF	:	Brain derived neurotrophic factor
BM	:	Bone marrow
BM-MSCs	:	Bone marrow derived mesenchymal stem cells
CD	:	Cluster of differentiation
CM	:	Conditioned Medium
DM	;	Diabetes Mellitus
DMEM	:	Dulbecco's modified Eagle's medium
EDTA	:	Ethylene diamine tetraacetic acid
EGF	:	Epidermal growth factor
EV	:	Extracellular vesicles
FBS	:	Fetal bovine serum
FGF-2/Bfgf	:	Fibroblast growth factor 2/basic fibroblast growth factor
GLUT2	:	Glucose transporter type 2
GLUT4	:	Glucose transporter type 4
HEGF	:	Heparin binding epidermal growth factor
HGF	:	Hepatocyte growth factor
hUCB-MSCs	:	Human umbilical cord blood derived mesenchymal stem cells
hUC-MSCs	:	Human umbilical cord derived mesenchymal stem cells
IGF-I	:	Insulin derived growth factor I
IGF-II	:	Insulin derived growth factor II

List of Abbreviations (Cont.)

Abb.:		*Full term*
KGF/FGF-7	:	Keratinocyte growth factor/ fibroblast growth factor 7
MHC-II	:	Major histocompatibility complex II
MNCs	:	Mononuclear cells
MODY	:	Maturity onset diabetes of the young
MSCs	:	Mesenchymal stem cells
MSCs-CM	:	Mesenchymal stem cells-conditioned medium
NGF	:	Neural growth factor
NPH-insulin	:	Neutral protamine Hagedorn insulin
PARP	:	Poly (adenosine-diphosphate-ribose) polymerase
PBS	:	Phosphate buffered Saline
PDEGF	:	Platelet derived endothelial cell growth factor
PDGF	:	Platelet derived growth factor
PDX1	:	Pancreatic/Duodenal homebox factor 1
PlGF	:	Placenta growth factor
SPSS	:	Statistical Package for the Social Sciences
STZ	:	Streptozotocin
T1DM	:	Type 1 Diabetes Mellitus
T2DM	:	Type 2 Diabetes Mellitus
UCB-MSCs	:	Umbilical cord blood derived mesenchymal stem cells
UC-MSCs	:	Umbilical cord derived mesenchymal stem cells
VEGF	:	Vascular endothelial growth factor

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ABSTRACT

Background: Diabetes Mellitus (D.M.) is a major health problem affecting more than 200 million worldwide. Type I diabetes mellitus (TIDM) is an autoimmune disease mediated by the destruction of β cells in the pancreas that has no definitive cure till present. Currently, regenerative medicine using umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) offers promising treatment. Meanwhile, Conditioned medium (CM) shows effectiveness for medication of various diseases.

Aim of the work is to compare the role of UCB-MSCs versus their CM alone on pancreatic beta cells in a rat model of Streptozotocin (STZ)-induced type I diabetes Mellitus.

Material and Methods: Forty adult male albino rats were divided randomly into 4 groups; **Group I (control group), Group II (Diabetic group)** which were injected (I.P) by a single dose of 1ml of STZ 35 mg/kg body weight and subdivided equally into **Subgroup IIA and Subgroup IIB** in which rats were sacrificed after 2 and 4 weeks respectively. **Group III (Diabetic + UCB-MSCs)** which were given STZ as in group II and each rat was injected with 1×10^6 cells/ml of UC-MSCs into tail vein and subdivided equally into **Subgroup IIIA and Subgroup IIIB** in which rats were sacrificed after 2 and 4 weeks respectively. **Group IV (Diabetic + CM)** which were given STZ as in group II and the rats received a dose of 0.5 ml of CM that was injected intramuscularly once per week and subdivided equally into **Subgroup IVA and Subgroup IVB** in which rats were sacrificed after 2 and 4 weeks respectively. Pancreatic specimens were prepared for histological and immune-histochemical techniques. Morphometrical and statistical studies were done.

Results: **Group II (Diabetic group)** stained by H& E showed distortion of the architecture of islets of Langerhans and multiple injuries in cells of the islets including vacuolations in the cytoplasm and small and darkly stained nuclei. In addition, it resulted in decrease size of islets and appearance of many empty spaces within it. There was significant decrease in body weight, serum insulin and C-peptide level and also, in insulin immunohistochemical stained positive cells. Moreover, significant increase in blood glucose and in caspase-3 immunohistochemical stained positive cells was found. **Group III (Diabetic + UCB-MSCs)** and **Group IV (Diabetic + CM**) both showed an obvious histological and biochemical improvement when compared to **Group II (Diabetic group).**

Conclusion: UCB-MSCs injection was more effective than injection of CM in the treatment of type I diabetes mellitus. However, CM represent a new modality of cell free therapies with broad application which need more investigations.

Keywords: Type I Diabetes mellitus, Umbilical cord blood derived mesenchymal stem cells, conditioned medium.

Introduction

Diabetes Mellitus (DM) type I known as Insulin-dependent Diabetes became very frequent chronic health condition in young adolescent population (**Praveen et al., 2016**). This disease can present long -term complications and the cause of high morbidity and mortality with impact on the quality of life. Patient with type I DM faces daily challenges in maintaining adequate blood glucose levels (**Guay et al., 2013**). The number of hospitalized patients due to onset of serious complications such as diabetic ketoacidosis and severe hypoglycemia has been increased **(Seth et al., 2015).** Type I Diabetes Mellitus is characterized by absolute insulin deficiency secondary to T cell mediated autoimmune destruction of pancreatic Beta cells **(Ilonen et al., 2019).**

Streptozotocin (STZ) is a widely used chemical for experimental induction of Diabetes Mellitus in animals **(Furman et al., 2015).**

Mesenchymal stem cells are multipotent stem cells that can be isolated from bone marrow, adipose tissue, umbilical cord and many other tissues. They could be used in regenerative medicine as they have the ability to renew themselves, differentiate into a wide range of cells and have high potentiality to expand in culture **(Fu et al., 2019)**. In addition, they have angiogenic, anti-apoptotic, anti-inflammatory, and immunomodulatory effects **(Xie et al., 2020).**

Umbilical cord was previously considered as biological waste; however, it has become an accepted source of human stem cells like those found in peripheral blood and bone marrow.

Umbilical cord stem cells possess many advantages over bone marrow stem cells for transplants. First, Umbilical cord stem cells processing, and collection is much easier and simpler. Indeed, the cord blood harvesting is quick and easy **(Alatyyat et al., 2020).**

Stem cells was found to repair tissues through paracrine mechanisms by expressing trophic and immunomodulatory factors. These trophic factors such as growth factors, anti-apoptotic, immunomodulatory and angiogenic factors would be capable to regenerate the injured tissues even if the stem cells didn't home in them. Another mechanism of repair was through their homing and differentiation into cells of damaged organ (**Omar et al., 2017).**

However, studies on stem cells-derived secreted growth factors showed that these factors alone without stem cells may repair tissues in various conditions of damage (**Timmers et al., 2011)**. Stem cells secreted these growth factors in the culture medium, so it was called conditioned medium (CM) **(Kim et al., 2013).**

The CM has many advantages compared to the use of stem cells as it can be manufactured, freeze dried packaged and transported more easily. Moreover, it is devoid of cells, so there's no need to match the donor and the recipient to avoid rejection problems **(Bogatcheva et al., 2019).**