VOLUME - 10, ISSUE - 12, DECEMBER - 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Physiology

HYPERGLYCEMIA REVERSAL IN DIABETIC INFARCTED RAT POST-INTRAVENOUS INFUSION OF HUMAN MESENCHYMAL STEM CELLS

Sara Jabeen	Department of Physiology, NIMS Medical College And Hospital, NIMS University, Jaipur- 303121, India
Usha Gupta	Department of Physiology, NIMS Medical College And Hospital, NIMS University, Jaipur- 303121, India
Aleem Ahmed Khan*	Scientist & Head Central Laboratory For Stem Cell Research & Translational Medicine, CLRD, Deccan College of Medical Sciences, Hyderabad, Telangana, India. *Corresponding Author

ABSTRACT

INTRODUCTION Hyperglycemia reversal and preservation/restoration of β-cells function in diabetic infarction remains as an attractive and challengeable therapeutic target. Mesenchymal stem cells (MSCs) are multipotent cells with a strong immunoregulatory potential that have emerged as a possible cell-based therapy for a variety of immunological diseases. The objective of this study was to examine the dose-dependent efficacy of intravenous administration of human umbilical cord blood derived MSCs (UCB-MSCs) in chemically induced rats with diabetic infraction.

METHODS Wister rats (weight: 200-250g, males) received intraperitoneal streptozotocin injection followed by isoproterenol to develop diabetes infarction condition. After model development animals received intravenous single or double dose of human UCB-MSCs (5 X 10^6 cells per animal at each dose) and followed up to 30 days post-administration. Pancreatic tissue histology, blood glucose and insulin levels were measured, and proportion of animal survival was calculated using Kaplan-Meier curve analysis.

RESULTS Double dose of MSCs infusion resulted in reorganization of islet cells and partial restoration of β-cells at day 30. Comparatively faster restoration of glucose and insulin normalization was observed for two MSCs doses compared to single dose. Highest proportion of animal survival was observed (>85%) for double doses of MSCs infusion compared to single dose (>70%) at day 30.

CONCLUSION Two consecutive intravenous doses of human UCB-MSCs can improve structural and functional deficits of pancreatic tissues and maintain blood glucose and insulin levels in diabetic infarcted rats up to 30 days. However, identification of long-term effects entails longer follow-up periods, and larger sample sizes with other investigations.

KEYWORDS : Human umbilical cord blood, mesenchymal stem cells, diabetes, myocardial infarction, β-cells

INTRODUCTION

Diabetic myocardial infarction is a critical complication observed in patients with uncontrolled and long-standing diabetes. A combination of glycemic control, and non-steroidal anti-inflammatory drug treatment provides short-term symptom resolution and reduced risk of recurrence. Diabetes is on the rise all over the world possibly due to population growth, ageing, rising obesity rates, and sedentary lifestyles. According to the International Diabetes Federation, diabetes affects at least 382 million people worldwide, with that number expected to rise to 592 million by 2035 (**IDF, 2013**). India has 62.4 million diabetics and 77.2 million persons with prediabetes, accounting for about one-fifth of the world's diabetic population (**Anjana et al. 2011**). This increasing trend in diabetes incidence worsens the pathophysiology of myocardial infarction (MI) resulting in poor clinical outcome.

To cure diabetes individually irrespective of myocardial infarction, current studies have focused on recovery or regeneration of the β-cell dysfunction and/or mass, regardless of the type of diabetes. However, the therapy's primary drawbacks include a scarcity of donors, a steady loss in insulin independence, and graft rejection. Stem cells, which can develop into insulin-producing cells *in vitro* or *in vivo*, would provide a potentially limitless source of islet cells for the treatment and alleviate the major hurdles of allogenic pancreatic islet availability and rejection (**Naftanel and Harlan, 2004; Berney et al. 2011; Bottino et al. 2018**).

Mesenchymal stem cells (MSCs) therapy has recently received a lot of attention due to its wide range of sources including adipose tissue, peripheral blood, dental pulp, bone marrow, and neonatal tissues, particularly in the placenta, and umbilical cord. Among all these sources umbilical cord blood (UCB) has been the most widely accepted source due to less ethical concern, ease of isolation, no chance of graft versus host diseases, no need of matching, and most importantly it is a biomedical waste (**Lakkireddy et al. 2021**). Further MSCs have several crucial advantages over other types of cells such as paracrine signalling, ability to differentiate into tissue specific cells, and endogeneous activation of resident stem cells to enhance repair and regenerative processes in damaged organs or tissues (**Guo et al. 2020**).

However, for a variety of reasons, the progress of MSCs therapy from bench to bedside has been extremely slow (**Wang et al. 2021**). The amount and quality of MSCs are the most difficult to work with in a clinic setting. Despite the fact that the process of isolating MSCs and expanding them into a nonclonal population of stromal cells has been standardized by the International Society for Cell & Gene Therapy (ISCT), MSCs from different donors or even different tissues have different proliferation rates and capabilities. Meanwhile, each nonclonal population of MSCs may have a variable fraction of stem cells, affecting the population's biological features. As a result, before MSCs are employed in patients, the percentage of stem and progenitor cells in each batch must be determined precisely. In terms of quality, MSCs ex vivo expansion eventually leads to cell senescence, which reduces MSCs capabilities, including differentiation, migration, and regeneration.

A growing number of investigations into the therapeutic potential of MSCs are now underway. MSCs have been shown to slow the course of diseases including myocardial infarction (**Ghanta et al. 2020**) and are capable of lowering blood glucose levels in diabetic condition (**Bhansali et al. 2015**). These findings show that MSCs can help enhance islet function, implying that MSCs therapy could help with hyperglycemia recovery. However, hyperglycemia reversal in diabetic infarcted setting using human UCB derived MSCs (UCB-MSCs) has not been demonstrated yet. Hence, in this

study we have tested the efficacy of single and double dose of human UCB-MSCs intravenous administration for hyperglycemia reversal and islet cells regeneration in diabetic infarcted rats.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Ethics Committee (IAEC), Deccan College of Medical Sciences, Hyderabad. The study procedures were conducted according to the guidelines for the Care and Use of Laboratory Animals. Male Wister rats of 180-200g were obtained from the National Institute of Nutrition (NIN), Hyderabad and housed at 24±4°C temperature, 55±5% humidity and at 12h dark light cycle while allowing free access to standard rat chow and drinking water.

Sample collection

After approval of Institutional Review Board, a total of five human umbilical cord blood (hUCB) samples were obtained from the Department of Gynecology, Owaisi Hospital and Research Centre, Hyderabad, Telangana, India. Informed consent forms were obtained from each participant.

Mononuclear cells isolation

Five milliliters (mL) of human UCB was diluted with 1X phosphate buffer saline (PBS, Catalog #: 10010023 Thermo Fisher Scientific) in 1:1 ratio and layered carefully on top of the Ficol paque density gradient medium (1.073 g/mL, HiSep™ LSM 1077, Catalog #: LS001, Himedia). The tube was centrifuged at 1100 × g/min for 30-40min and mononuclear cells (MNCs) were collected from the interface layer, suspended in two volumes of PBS, and collected by centrifugation at 1100 × g/min.

Cell counting and viability testing

Viability and count of MNCs harvested from each sample was determined using Trypan Blue Exclusion Assay with the help of hemocytometer. Briefly, cells were diluted 1:100 with PBS and mixed with 0.4% Trypan Blue dye and incubated at room temperature for 5min. Further 20μL of cells with dye was loaded onto a hemocytometer and covered with cover slip. Number of viable and dead cells were counted in each chamber and percentage viability and cell count was calculated using following formula.

Percentage viability = (Number of viable cells/Total number of cells) X 100
Viable cells/mL = (Total number of viable cells counted in 4 squares/4) X 10,000

In vitro enrichment of human MSCs

In vitro enrichment of MSCs was performed by culturing harvested MNCs on plastic surfaces under controlled condition. Briefly, MNCs were mixed with low glucose Dulbecco's Modified Eagle Medium (DMEM-LG; Gibco™ 31885049, Thermo Fisher Scientific) which were supplemented with heat inactivated 10% fetal bovine serum (Gibco® Qualified FCS, 26140087, Thermo Fisher Scientific), and 1X antibiotic and antimycotic solution (Catalog #: 15240062, Thermo Fisher Scientific). Furthermore, 2 X 10^4 cells were seeded into a T-25 flask and cultured at 37°C with 95% humidity in a 5% CO_2 atmosphere. After the third day, fresh culture medium was added, and floating or non-adherent cells were removed. Cells were given the opportunity to grow for 21 days. After reaching 80% confluency, cells were taken from the culture flask using 0.25% trypsin (Catalog #: 93595, Sigma) at each passage and sub-cultured at a 1:3 ratio to expand up to 6 passages.

Growth rate analysis of human MSCs

For all five samples, MSCs growth rates were measured using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) cell proliferation test from day 1 to day 21 at first passage and from day 1 to day 14 at successive passages. Briefly, 2mg/mL MTT was added to each sample parallel grown in 96 well plates in triplicates at each time point and incubated for 4 hours at 37°C. Following incubation, dark blue formazan crystals generated in actively metabolising cells were dissolved in dimethyl sulfoxide (DMSO, Sigma Catalog #: D2650), and optical density was measured using a microplate reader at 570nm wavelength.

MSCs characterization

Each enriched MSC population was identified using morphometric appearance through microscopic imaging, and viability was determined in a time-dependent manner using the MTT (Catalog #: M2003, Sigma) tetrazolium reduction assay. The positive markers CD73, CD90, and CD105, as well as the negative marker CD34, were quantified using a SYBR Green-based real-time quantitative polymerase chain reaction (RT-qPCR) method (S1000, Bio-Rad, USA).

Briefly, total ribonucleic acid (RNA) was isolated from cells on days 1, and day 14 using the guanidium isothiocyanate (GITC, Catalog #: 50983, Sigma) method and transformed to complementary DNA (cDNA) using MMLV reverse transcriptase II (Thermo Fisher Scientific, Catalog #: 28025013). RT-qPCR was performed in triplicates using 5ng cDNA and GAPDH specific primers as an endogenous control as described in our earlier studies (**Jabeen et al. 2021; Lakkireddy et al. 2021**).

Animal model development

Wister rats weighing 280 to 300g were divided into four groups: 1) Control, 2) diabetes+MI receiving saline, 3) diabetes+MI receiving single dose of MSCs, and 4) diabetes+MI receiving double dose of MSCs. Each group included a total of twelve (12) rats. The rats in group 2 were given 5 doses of streptozotocin (STZ, 40 mg/kg body weight), while in group 3 additional 2 doses of isoproterenol (85 mg/kg) through intraperitoneal injections were given after attaining a fasting blood glucose level of more than 200 mg/dL. Infarction was confirmed by estimation of Troponin 1 and Glutathione levels.

Dose-dependent infusion of hMSCs *in vivo*

Human MSCs (5 X 10^6 cells per animal at each dose) were delivered intravenously to diabetic infracted rats (n=12) as single dose at day 1 and double-dose (at day 1 and day 7). At day 30 after MSCs infusion, the alterations in pancreatic histology, and blood glucose and insulin levels were examined.

Histological analysis

At day 30 after MSC infusion, Hematoxylin and Eosin (H&E) staining was performed for rat pancreatic tissues. Briefly, rats were slaughtered, and tissues were dissected, and paraffin blocks were created. Using a cryotome, tissues were sliced to a thickness of 3-5μm. In all groups, each segment was fixed in 10% buffered formalin and then processed for H&E staining. Inverted fluorescence microscopy images were documented using Axiovert software (version 4, Carl Zeiss, Germany). A pathologist reviewed all of the images and presented his results in a blinded manner.

Estimation of blood glucose level

Blood glucose level was estimated in from each animal after 6 hours of fasting before and after MSCs infusion at different time points up to day 30. A commercially available and clinically authorized glucometer (GlucoChek, India) with a range of 0–600 mg/dL was used to estimate glucose levels. Using a lancet, a small drop (2-4mL) of blood was drawn from rat's tail vein and utilized to test the glucose level of the animals using disposable glucose measuring strips (GlucoChek, India). Rats were classified as normoglycemic if their fasting glucose level was less than 200 mg/dL after 6 hours of fasting. Strips were coded for each group of animals before testing. Each time, a single strip was used for testing.

Estimation of blood insulin level

The levels of insulin in the blood was measured using a mouse/rat insulin ELISA kit (Cat.: EMINS, Thermo Scientific, USA). Before processing, all the samples were brought to room temperature and working solutions were prepared in accordance with the manufacturer's standards. 100μL of each standard and test sample were transferred to the respective wells in triplicate. The plate was covered properly and incubated overnight at 4°C with gentle shaking. The well was rinsed with 1X wash buffer after the solutions were aspirated. Each well received 100μL of 1X biotinylated antibody, which was incubated at room temperature for 60min with gentle shaking. After that, 100μL of streptavidin-HRP solution was added and incubated for another 45min. Each well was washed after the solution was aspirated. 100μL of TMB substrate was added in each well and incubated at room temperature for 300min. Within 30min of terminating the procedure, the absorbance was measured at 450–550nm. Interpolating the absorbance with insulin concentration in relation to standards was used to estimate insulin in each test sample. To determine the actual concentration of insulin in the original sample, the resultant value was multiplied by the sample dilution factor.

Animal survival

Animal survival with or without MSCs administration of single or double dose in all four group of animals was assessed through the Kaplan-Meier curve analysis up to 30 days. The proportion of animal survival in each group was recorded and represented using Kaplan-Meier curve.

Statistical analysis

All the values were presented as mean±standard deviation (SD). Student t-test and one-way ANOVA was used to compare different groups for producing statistical significance. *p* value less than 0.05 was set to determine statistical significance for all the variables.

RESULTS

Enrichment and growth rate of human UCB-MSCs

Enrichment of human UCB-MSCs was first confirmed by morphometric assessment under the microscopy from day 1 to day 21. The microscopic observation showed changes in cellular morphology from spherical to spindle shape from day 1 to day 21 (**Figure 1A**). The Highest confluency of spindle-shaped cells was observed at day 14. Further, MTT growth rate analysis showed significant increase in cell number from day 1 to day 7, day 14, and day 21 ($p<0.0001$, **Figure 1B**). Characterization of human MSCs using RT-qPCR showed significantly higher fold values for positive markers (CD90, CD73 and CD105) whereas non-significant expression levels were observed for CD34 (**Figure 1C**).

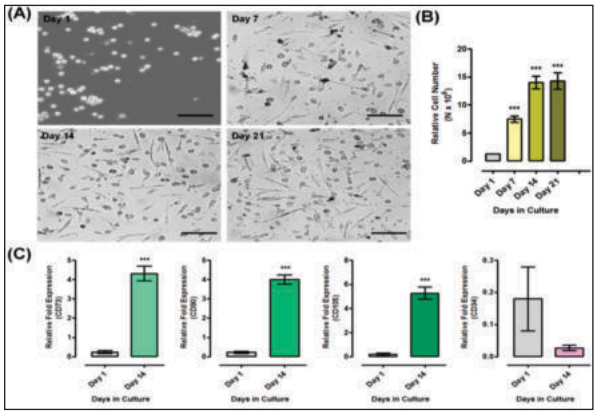

Figure 1: MSCs enrichment, growth rate and characteristics. (A) Morphometric analysis, **(B)** MTT growth rate analysis, and **(C)** Molecular characterization of MSCs. ***p<0.0001

Diabetic-infarcted animal models

Establishment of diabetic-infarcted animal models in rats was confirmed by significantly increased levels of troponin 1 ($p<0.0001$) and significantly decreased levels of glutathione ($p<0.0001$) compared to controls (**Figure 2**). Further, histological analysis of pancreatic tissues also confirmed establishment of diabetes model which was identified through loss of islet cells (**Figure 3**), and damage to cardiac tissues (data not shown).

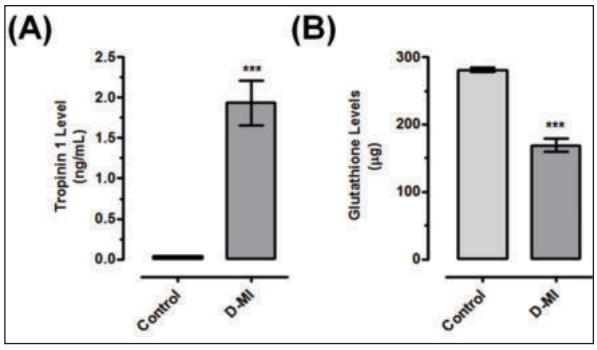

Figure 2: Myocardial infarction in diabetic rats was identified using levels of (A) Tropinin1 and (B) Glutathione. ***p<0.0001

Improvement in diabetic-infarcted rats after MSCs infusion

Improvement in diabetic-infarcted rats after MSCs infusion was determined through qualitative assessment of pancreatic tissue histology, and quantitative assessment of blood glucose and insulin levels. Proportion of animal survival was assessed using Kaplan-Meier curve.

Improvement in pancreatic tissue histology

Histological analysis of the pancreatic tissues after H&E staining showed destruction of islet tissue and low insulin reactivity on immunofluorescence in the diabetic-infarcted group compared to control group. MSCs infusion in diabetic-infarcted rats resulted in reorganization of islet and partial restoration of β-cells compared to the diabetic-infarcted rats at day 30 (**Figure 3**). Comparatively faster restoration and normalization of islet cells was observed for two MSCs doses compared to single dose.

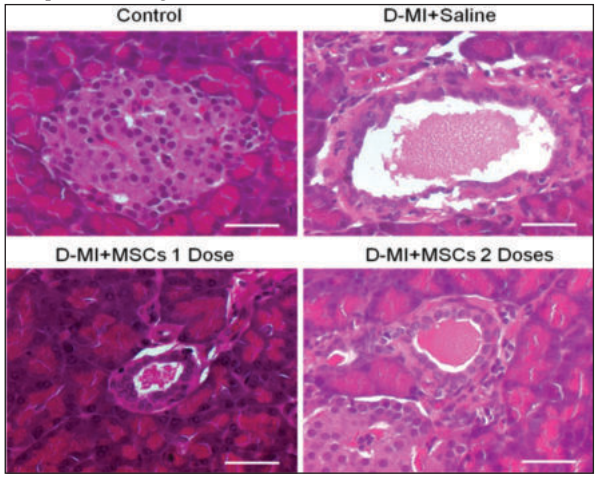

Figure 3: Changes in pancreatic tissue histology in diabetic infarcted rats after MSCs infusion at single and double dose

Restoration of blood glucose level

Fastest recovery in blood glucose levels was observed for MSCs 2 doses infusion compared to single dose. Double dose of MSCs resulted in complete normalization and stabilization of blood glucose levels at day 15 and maintained up to day 30 (**Figure 4**).

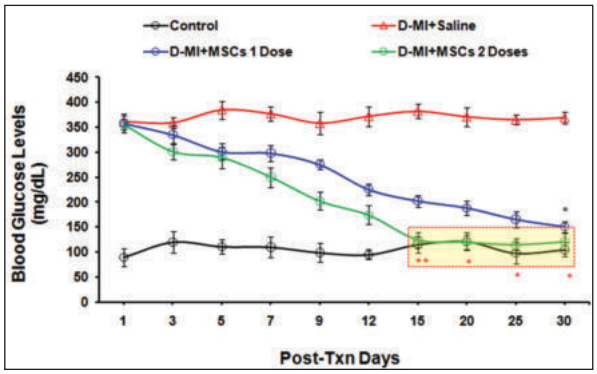

Figure 4: Changes in blood glucose levels in diabetic infarcted rats after MSCs infusion at single and double dose up to 30 days. *p<0.01, **p<0.001

Restoration of blood insulin level

Similar to blood glucose levels, fastest recovery in blood insulin levels was observed for MSCs 2 doses infusion compared to the single dose. Double dose of MSCs resulted in complete normalization and stabilization of blood insulin levels at day 5 and maintained up to day 30; whereas single dose of MSCs infusion didn't recover blood insulin levels (**Figure 5**).

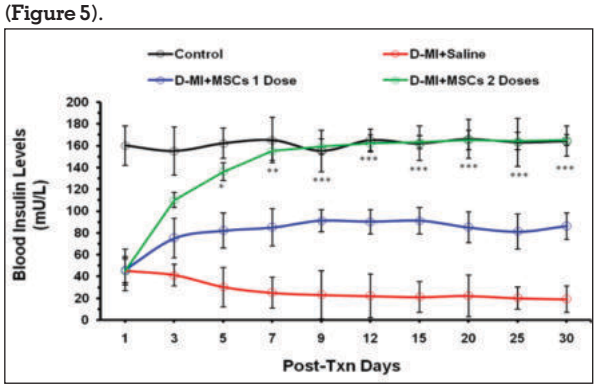

Figure 5: Changes in blood insulin levels in diabetic infarcted rats after MSCs infusion at single and double dose up to 30 days. *p<0.01, **p<0.001, ***p<0.0001

Animal survival

Kaplan-Meier curve analysis showed highest proportion of animal survival (>85%) for double doses of MSCs infusion compared to single dose (>70%) at day 30 which was significantly higher than diabetic-infarcted group (**Figure 6**).

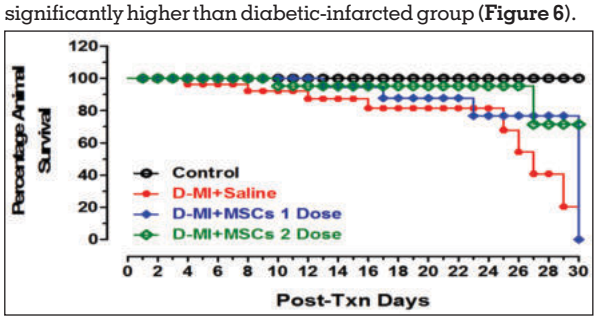

Figure 6: Kaplan-Meier plot showing proportion of survival of diabetic infarcted rats after MSCs infusion at single and double dose up to 30 days

DISCUSSION

In the present study, we have demonstrated following effects of allogenic human UCB-MSCs intravenous transplantation in diabetic infarcted rats: (1) significant improvement in pancreatic tissue histology; (2) normoglycemia; (3) insulin optimization; and (4) improvement in animal survival.

Earlier studies have demonstrated that MSCs transplantation exerts improvement in glycemic profile of hyperglycemic animals (**Tsai et al. 2012; Aali et al. 2014; Bhansali et al. 2015**). Similarly, several other studies have also reported improvement in conditions like diabetic nephropathy and cardiac functions after transplantation of MSCs from different sources (**Li et al. 2018; Guo et al. 2020**). However, there is scarcity of data showing glycemic reversal in diabetic infarcted condition using human UCB-MSCs.

MSCs are one of the most prominent multipotent adult stem cells. MSCs hold great promise for treating disease and regeneration of injured tissues, due to their abilities to differentiate into replacement cells in injured tissues, modulate their local environment, activate endogenous progenitor cells, and secrete various factors (**Ankrum and Karp, 2010; Si et al. 2011**). Despite this, the precise mechanisms underlying these impacts remain unknown. Because only a small percentage of MSCs can develop into functionally competent β-cells *in vivo* (**Ianus et al. 2003**), it seems likely several other mechanisms underlie MSCs' therapeutic benefit in diabetes. MSCs have been demonstrated to release a number of trophic cytokines that improve the pancreas microenvironment and encourage the growth of endogenous pancreatic stem cells (**Lee et al. 2006; Park et al. 2010**). However, these findings are still not adequate to explain the therapeutic contribution of MSCs to diabetic infarcted condition.

In this study, we have reported structural and functional improvement in pancreatic tissues and regeneration of islet cells at day 30 with two consecutive doses of MSCs intravenous infusion in diabetic infarcted animals. The probable reason for such improvement can be explained through above mechanisms of paracrine effects, endogeneous activation of resident progenitor cells and transdifferentiation of MSCs into insulin producing cells (**Park et al. 2010**). Further, immunomodulatory properties of MSCs provides additional advantages over other types of cells by providing suitable microenvironment to induce endogenous activation and proliferation of cells without eliciting significant immunological responses (**Wang et al. 2021**).

We could achieve hyperglyecemia reversal at day 15 post-infusion of MSCs. Likewise, insulin level was also recovered and maintained in long-term (up to 30 days) with two consecutive doses of MSCs. These data clearly show that multiple doses of MSCs infusion exerts faster and longer beneficial effects in disease conditions than single dose. However, hypoglycemia is another challenge observed after multiple doses of cellular transplantation (**Hu et al. 2016; Bhansali et al. 2017**), which is more critical than hyperglycemia resulting in early morbidity and mortality. Thus, in this study, we compared single and double doses of MSCs infusion to reverse hyperglycemia in diabetic infarcted animals. And double doses of MSCs provided best response without the risk of hypoglycemia up to 30 days. The highest proportion of animal survival (>85%) for double doses of MSCs infusion was observed compared to single dose (>70%) at day 30 which was significantly higher than diabetic-infarcted group. In accordance, a recent clinical study has demonstrated clinical remission up to 12 months in patients treated with MSCs which was 2.5-fold higher than the control group, indicating preservation of β-cells functional efficacy (**Lu et al. 2021**).

Despite promising outcomes in pre-clinical investigations, there are a number of difficulties that must be addressed before MSC-based therapies may be deemed a safe and effective choice for clinical trials. Cell source, treatment cycle, culture expansion methodology, passage number, timing and route of administration, dosage, donor features, freshly

generated, or cryopreserved cells are all factors that influence MSCs clinical success. Currently, the clinical application cycle of MSCs is difficult to unify, and there is no universally accepted therapy principle. Varied hospitals and laboratories have vastly different diagnostic and treatment options.

CONCLUSION

Two consecutive intravenous doses of human UCB-MSCs can improve structural and functional deficits of pancreatic tissues and maintain blood glucose and insulin levels in diabetic infarcted rats up to 30 days. However, identification of long-term effects of MSCs entails longer follow-up periods, and larger sample sizes with other investigations.

Declaration of Competing Interest: The authors report no declarations of interest.

Acknowledgements: None

REFERENCES

1. International Diabetes Federation (IDF). IDF diabets atlas, 6th ed. Brussels, Belgium: IDF; 2013.
2. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. Diabetologia 2011; 54 : 3022-7.
3. Naftanel MA, Harlan DM. Pancreatic islet transplantation. PLoS Med. 2004;1(3):e58-e75.
4. Berney T, Korsgren O, Posselt A, Pileggi A. Islet Transplantation & □-Cell Replacement Therapies for Diabetes. Journal of Transplantation, 2011; 1578401-2.
5. Bottino R, Knoll MF, Knoll CA, Bertera S and Trucco MM (2018) The Future of Islet Transplantation Is Now. Front. Med. 5:202. doi: 10.3389/fmed.2018.00202.
6. Lakkireddy C, Vishwakarma SK, Raju N, et al. Fabrication of Decellularized Amnion and Chorion Scaffolds to Develop Bioengineered Cell-Laden Constructs. Cel. Mol. Bioeng, 2021.
7. Guo Y, Yu Y, Hu S, Chen Y, Shen Z. The therapeutic potential of mesenchymal stem cells for cardiovascular diseases. Cell Death and Disease, 2020; 11:349.
8. Wang Y, Shan S-K, Guo B, Li F, Zheng M-H, Lei L-M, Xu Q-S, Ullah MHE, Xu F, Lin X and Yuan L-Q. The Multi-Therapeutic Role of MSCs in Diabetic Nephropathy. Front. Endocrinol. 2021; 12:671566.
9. Ghanta RK, Aghlara-Fotovat S, Pugazenthi A, Ryan CT, Singh VP, Mathison M, et al. Immune-Modulatory Alginate Protects Mesenchymal Stem Cells for Sustained Delivery of Reparative Factors to Ischemic Myocardium. Biomater Sci, 2020; 8(18):5061–70.
10. Bhansali S, Dutta P, Kumar V, Yadav MK, Jain A, Mudaliar S, et al. Efficacy of autologous bone marrow-derived mesenchymal stem cell and mononuclear cell transplantation in type 2 diabetes mellitus: a randomized, placebo-controlled comparative study. Stem Cells Dev. 2017; 26(7):471–81.
11. Tsai PJ, Wang HS, Shyr YM, Weng ZC, Tai LC, Shyu JF, Chen TH. Transplantation of insulin-producing cells from umbilical cord mesenchymal stem cells for the treatment of streptozotocin-induced diabetic rats. Journal of Biomedical Science, 2012; 19:47.
12. Aali E, Mirzamohammadi S, Ghaznavi H, Madjd Z, Larijani B, Rayegan S, Sharifi AM. comparative study of mesenchymal stem cell transplantation with its paracrine effect on control of hyperglycemia in type 1 diabetic rats. Journal of Diabetes & Metabolic Disorders, 2014; 13:76.
13. Jabeen S, Gupta U, Khan AA. Cellular and molecular evaluation of in vitro quality parameters of human umbilical cord blood-derived mesenchymal stem cells. Ind J App Res, 2021; 11(11):52-54.
14. Li Y, Liu J, Liao G, Zhang J, Chen Y, Li L, et al. Early intervention with mesenchymal stem cells prevents nephropathy in diabetic rats by ameliorating the inflammatory microenvironment. International Journal of Molecular Medicine, 2018; 41:2629-2639.
15. Ankrum J and Karp JM. Mesenchymal stem cell therapy: two steps forward, one step back. Trends Mol Med, 2010;16:203–209.
16. Si YL, Zhao YL, Hao HJ, Fu XB, Han WD. MSCs: biological characteristics, clinical applications and their outstanding concerns. Ageing Res Rev, 2011; 10:93–103.
17. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest, 2003;111:843–850.
18. Lee RH, Seo MJ, Reger RL, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. PNAS, 2006; 103:17438–17443.
19. Park KS, Kim YS, Kim JH, et al. Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation. Transplantation, 2010; 89:509–517.
20. Hu JX, Wang YG, Gong HM, Yu C, Guo C, Wang F, et al. Long term effect and safety of Wharton's jelly-derived mesenchymal stem cells on type 2 diabetes. Exp Ther Med. 2016; 12(3):1857–66.
21. Lu J, Shen S, Ling Q, Wang B, Li L, Zhang W, Qu D, et al. One repeated transplantation of allogeneic umbilical cord mesenchymal stromal cells in type 1 diabetes: an open parallel controlled clinical study. Stem Cell Research & Therapy, 2021; 12:340.