Secretome of Hypoxia-Preconditioned Mesenchymal Stem Cells Ameliorates Hyperglycemia in Type 2 Diabetes Mellitus Rats

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Abstract

Introduction: Type 2 diabetes mellitus (T2DM) is a prevalent form of diabetes that affects 90 - 95 % of all diabetic patients. Insulin sensitizers and insulin exogenous supply could temporarily ameliorate hyperglycaemia; however, they are accompanied by side effects. As a result, new approaches are required to address insulin resistance and regenerate beta cells simultaneously. The secretome of hypoxic mesenchymal stem cells (SH-MSCs) contains various growth factors and anti-inflammatory cytokines that could potentially enhance insulin resistance and improve pancreatic function. Objectives: In this study, we performed SH-MSCs infusion to ameliorate HFD-induced hyperglycaemia in T2DM rats. Methods: We created a T2DM rat model using a combination of a high-fat diet (HFD) and streptozotocin (STZ) administration. Then, we administered SH-MSCs injection at doses of 250 and 500 µL and assessed the therapeutic effects of SH-MSCs. We also investigated the potential underlying mechanisms involved. Results: The administration of SH-MSCs improved hyperglycemia in rats with T2DM. Infusion of SH-MSCs at 500 µL dose decreased homeostatic model assessment for insulin resistance (HOMA-IR). Histological analysis revealed that injection of SH-MSCs alleviated morphological damage of pancreas. SH-MSCs administration also inhibit the level of IL-6 and promote the expression of CD163 type 2 macrophage. Conclusion: The results of our study indicate that SH-MSCs have the potential to improve hyperglycemia and exert a protective effect on T2DM rats.

Keyword: T2DM, SH-MSCs, Hyperglycaemia, Insulin resistance

Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes and affects 90 - 95 % of all diabetic patients [1]. In 2015, around 3.4 million people were diagnosed with diabetes, and this number increased by 15.2 % in 2019 [2]. The T2DM primary etiologist are abnormal glucose homeostasis, insufficient insulin secretion, insulin resistance, and a decrease in pancreatic β-cell function. Persistent hyperglycaemia can lead to β-cell function exhaustion because of deterioration of insulin resistance, and eventually cause complications [3]. Insulin sensitizers and insulin exogenous supply as current medications for T2DM could temporarily ameliorate hyperglycaemia, however they are accompanied by side effects and could improve neither insulin resistance nor progressive β-cell dysfunction [4]. Consequently, new therapeutic strategies are necessary to treat T2DM patients by repairing insulin resistance and regenerating β-cells.

Mesenchymal stem cells (MSCs) secrete a variety of bioactive molecules, collectively referred to as the secretome, that contribute to their therapeutic effects. Secretome profiles can be increase by exposing MSCs to specific pre-condition stimuli including hypoxia, oxidative stress, growth factors, genetic

modification, cytokines, or exposure to certain chemicals [5]. Recent studies reported that MSCs in hypoxia condition possess the potency to express several growth factors and anti-inflammatory cytokines, including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) and interleukin-10 (IL-10) [6,7]. This combination of molecules, known as the H-MSCs secretome (SH-MSCs), could potentially improve insulin resistance and pancreatic function. MSCs are multipotent cells that can adhere to plastic culture and express several surface markers, including CD90, CD44, CD105, CD73, and CD29, while not expressing CD45, CD34, CD19, CD14, CD11b, or human leukocyte antigen (HLA)-DR [8-10]. Additionally, MSCs have the capacity to develop into various mature cell types, such as osteocytes, adipocytes, osteocytes, and nerve cells [11].

Previous studies reported that cell-based therapy using MSCs is a promising strategy to improve T2DM. Different sources of MSCs potentially improved the T2DM condition [8,12]. The infusion of MSCs from bone marrow ameliorated the hyperglycaemia and hyperinsulinemia in early and late phase of T2DM [8]. The use of MSCs from adipose tissue also restored the T2DM through the repairing the abnormal signalling transduction and proinflammatory milieu [13]. Other studies also revealed the capability of MSCs from umbilical cords and their exosomes in ameliorating insulin resistance in T2DM rats [14]. These studies suggested that MSCs significantly reduced insulin resistance in typical T2DM rats through their molecule secretion, including cytokines, growth factors and even exosomes. Previous study also reported that hypoxic secretome MSCs significantly increased the SOD gene expression associated with suppression of IL-6 level on T1DM [15]. However, the potency of molecules contained in SH-MSCs in ameliorating T2DM is still unclear.

In this investigation, we employed SH-MSCs that were effectively separated from H-MSCs using tangential flow filtration in accordance to the molecular weight cut-off (MWCO) category regarding our prior study [16]. We used a high-fat diet (HFD) along with the administration of streptozotocin (STZ) to induce T2DM in a rat model. We then performed SH-MSCs infusion at day 0, 7 and 14 after STZ injection ameliorating HFD-induced hyperglycaemia in T2DM rats.

Methods

Animals

The use of animals in this study was approved by the Institutional Animal Care and Use Committee at Universitas Islam Sultan Agung, under reference number 282/VIII/2022/Komisi Bioetik. we used 28 male Sprague-Dawley rats that were 8 weeks old and weighed 200 ± 20 g. Rats were acclimated for a week with a temperature of 25 ± 2 °C and a cycle of light and darkness lasting 12/12 h. Standard housing arrangements included ad libitum access to food and water for the animals. They were kept in cages under standard conditions.

Induction of T2DM

After acclimatization, the rats were divided into 2 groups. The normal control group was fed a regular pellet diet, while the other group was fed a high-fat diet (HFD) for 8 weeks. The HFD had the following ingredients: 5 % refined sugar, 2.5 % cholesterol, 2.5 % starch, 20 % refined cooking oil, 50 % ground standard pellet, and 20 % full cream milk powder. For every week, all of the rats' body weights were measured. Nicotinamide (120 mg/kg body weight; Sigma-Aldrich, MO, USA) and STZ (60 mg/kg body weight; Santa Cruz Biotechnology, TX, USA) were injected intraperitoneally (IP) to the non-fasting rats fed with the high-fat diet (HFD) at 15 min intervals. Once dissolved, the STZ solution was freshly prepared and employed within 5 min. As a control group, rats fed on a regular pellet diet were received 200 µL each of phosphate-buffered saline (PBS) and 0.05 M sodium citrate buffer (pH 4.5) instead of nicotinamide and STZ, respectively. The rats were subjected to a 6-hour fasting period after 7 days. To confirm the onset of DM, the fasting blood glucose, C-peptide and insulin levels were assessed. The diabetic rats employed in this investigation were those that had been fed a HFD and had elevated fasting blood glucose levels (>15 mmol/L). The following groups of T2DM rats (n = 6/group) were randomly assigned, and the treatment intervention was carried out for 21 days. Throughout the treatment period, the rats were kept on a HFD with exception of the normal control group. The diabetic rats received 250 and 500 µL SH-MSCs (every week, totalling 3 doses) through tail vein intravenous injection and the T2DM group only received the NaCl as a control vehicle.

Preparation, flow cytometry phenotyping and differentiation analysis of UC-MSCs

Under general anesthesia, umbilical cords (UCs) from Wistar rats that were 19 days pregnant were removed. According to our previous report, UC-MSC isolation was carried out [6]. The UC-MSCs were

cultured in a culture flask using Dubbelco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Louis st, MO, USA) along with 10 % fetal bovine serum (FBS, Gibco™ Invitrogen, NY, USA) 1.5 % penicillin/ streptomycin (Gibco™ Invitrogen, NY, USA) and 0.25 % amphotericin B from (Gibco™ Invitrogen) 37 °C and 5 % CO_2 were used to incubate the cells. The medium was changed every 3 days. MSCs were transferred into a fresh flask once they had reached 80 % confluence. For the subsequent studies, UC-MSCs from the 5th passage were used.

In accordance with the manufacturer's instructions, flow cytometry analysis was used to characterize the surface markers on UC-MSCs at the third passage. In brief, rat anti- CD90-FITC, CD29-PE, CD31-perCP, and CD45-APC antibodies (BD Bioscience, San Jose, CA, USA) were then incubated for 20 min in the dark at room temperature to stain the membrane antigens. Following the incubation, the cells were examined using a BD Accuri C6 Plus flow cytometer and analyzed using BD Accuri C6 Plus software (BD Bioscience).

We also examined the differentiation of MSCs in the 5th passage into adipogenic and chondrogenic tissues. Up to 95 % confluence, UC-MSCs were grown in standard media at 37 °C and 5 % CO_2. Then, the normal medium was changed with Rat MesenCult™ adipogenic and osteogenic differentiation basal media, respectively, supplemented with each supplement (Stem Cell Technologies, Singapore), 1 % L-glutamine, 1 % penicillin, and 0.25 % amphotericin B (Gibco). Every 3 days, the medium was changed. After 21 days of incubation, the lipid and calcium deposits were stained using oil red O and alizarin red staining (Sigma-Aldrich, Louis St, MO).

Induction of hypoxia in UC-MSCs

Using a hypoxic chamber (Stem Cell Technologies), hypoxia was produced in UC-MSCs when they had attained 80 % confluences and maintained at a concentration of 5 % O_2. Utilizing an oxygen controller, the oxygen partial pressure (pO2) value was verified (BioSpherix, Lacona, NY, USA). The cells were then incubated for 24 h at 37 °C with 5 % CO_2. After the incubation, the culture media was collected.

Preparation of SH-MSCs

After being centrifuged at 13,000 g for 10 min at 4 °C, the conditioned medium (CM) of UC-MSCs under hypoxia conditions was separated from the culture condition. According to Putra *et al.* [16], the separation of SH-MSCs was accomplished utilizing the tangential flow filtration (TFF) approach (Formulatrix, MA, USA). Utilizing 10 - 50 kDa 50 %, 50 - 100 kDa 25 %, and 100 - 300 kDa 25 % filter cassettes, the molecules from H-MSCs-CM were filtrated (Formulatrix). The SH-MSCs were then used in the subsequent experiment and kept at −80 °C for use in enzyme-linked immune sorbent assays (ELISAs).

SH-MSCs profilling

According to the manufacturer, the cytokine and growth factor levels in SH-MSCs were examined (Invitrogen, CA, USA). At room temperature, the ELISA for VEGF, PDGF, bFGF, IL-10, TGF, and IL-6 were employed. Using a microplate reader with a wavelength of 450, the data were examined (Bio-Rad, CA, USA).

Treatment of SH-MSCs

For this experiment, 6 animals per group were employed. The study was divided into 4 groups. NaCl was administered intravenously to T2DM and healthy control rats. On the treatment groups, T2DM rats were administered 250 and 500 µL SH-MSCs through the tail vein, respectively (every week, total 3 doses.

Blood glucose analysis

Blood glucose test was performed on the next week of the last treatment intervention. After overnight fasting blood glucose levels were determined using the Accu-Check Glucometer (Roche).

Analysis of serum parameters

A week after treatment, whole blood samples were collected. The level of IL-6, C-peptide and insulin in rat fasting serum were analysed using ELISA, according to manufacturer protocols (Invitrogen, CA, USA). The following formula was used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR):

$$HOMA - IR = \frac{\frac{Fasting\ blood\ glucose\ (\frac{mmol}{L})}{Fasting\ serum\ insulin\ (\mu U/mL)}}{22.5}$$

Histological analysis

One week following treatments, pancreatic tissue samples were collected. The samples were immediately fixed in cold 4 % neutral buffer formalin, at 4 °C, and processed for paraffin embedding. Before staining, sections were deparaffinized and rehydrated, using xylol and alcohol. Sections were then fixed with acetone for hematoxylin-eosin (H&E) staining using standard techniques.

Immunohistochemical examinations of CD163

Pancreatic slides that were embedded in paraffin were deparaffinized using xylene and alcohol. Slides were rehydrated before being exposed to a rat primary monoclonal antibody for CD163 (1:100, Abcam, Cambridge, MA, United States), which was followed by a biotinylated secondary antibody. Streptavidin peroxidase was used to assess the detection, and ImageJ was used to semi-quantify the level of CD163 expression.

Data analysis

The mean ± standard deviation (SD) was used to express all results. Utilizing the statistical program SPSS version 26.0 (SPSS Inc., Chicago, IL, USA), differences statistical significance was assessed. The findings were analysed using a one-way ANOVA and the least significant difference (LSD) post hoc test. A p-value below 0.05 ($p < 0.05$) was considered as statistically significant.

Results

The characteristics of UC-MSCs

We examined the cell morphology, membrane marker expression, and differentiation capacity at the 5th passage to carry out the characteristics of UC-MSCs, as recommended by the International Society for Stem Cell Therapy (ISCT). The isolated cells displayed a fibroblast-like spindle-shaped feature (**Figure 1A**). We conducted the adipogenic (**Figure 1B**) and osteogenic differentiation analysis (**Figure 1C**) to verify the UC-MSCs capacity for differentiation. Following a 21-day incubation period, the UC-MSCs were differentiated into adipocytes and osteocytes, which are distinguished by the deposition of lipid and calcium, respectively, marked as red color. The cells immunophenotyping profile also revealed that CD90.1 and CD29 were expressed positively whereas CD45 and CD31 were not (**Figure 1D**).
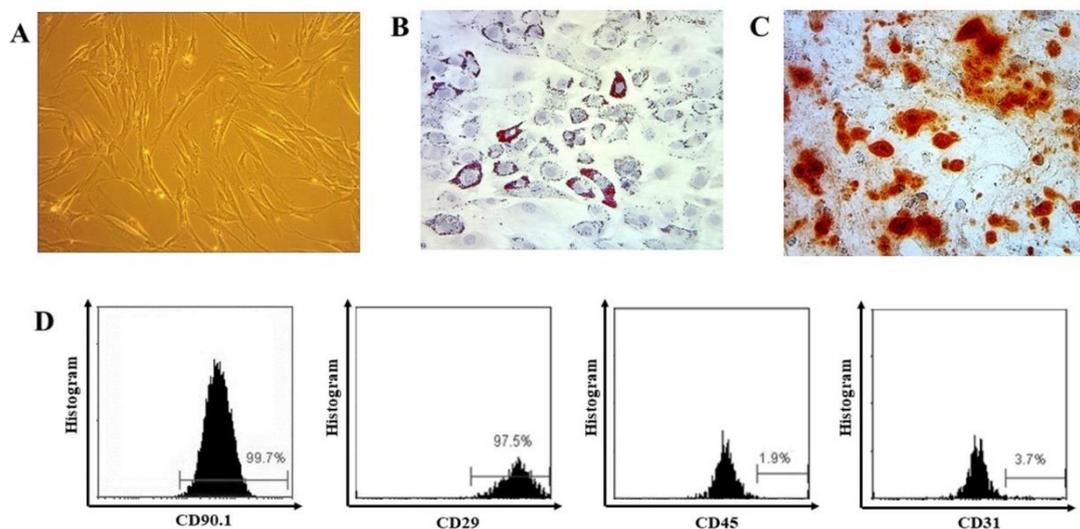

Figure 1 The characteristics of UC-MSCs. A) Under 200× magnification, UC-MSCs displayed spindle-shaped and fibroblast-like properties during the 5th passage. After being incubated with oil red O and alizarin red staining, respectively, UC-MSCs may differentiate into adipocytes (b) and osteocytes (c), which are identifiable by their red color (200× magnification). In the phenotyping examination of UC-MSCs, CD90.1 (99.7 %) and CD29 (97.5 %) were found to be positively expressed, whereas CD45 (1.9 %) and CD31 (3.7 %) were found to be negatively expressed.

The level of several molecules contained in SH-MSCs

We collected the CM from H-MSCs following the incubation of UC-MSCs in a hypoxia environment for 24 h. To acquire pure SH-MSCs, we separated the cytokines and growth factors present in H-MSC-CM. Based on a combination of molecular weight cut-off categories, we used the TFF strategy regarding our prior study [16]. We used 10 - 50 kDa 50 %, 50 - 100 kDa 25 %, and 100 - 300 kDa 25 % filter cassettes to isolate the molecules. After the filtration, we used the ELISA to evaluate the level of cytokines and growth factors present in SH-MSCs. The level of soluble molecules contained in SH-MSCs was presented in **Table 1**.

Table 1 Analysis of cytokines and growth factors of SH-MSCs. The analysis was employed using ELISA assay.

Molecules	SH-MSCs Value ± SE (pg/mL)
VEGF	1228.86 ± 27.71
PDGF	1043.06 ± 24.49
FGF	1085.34 ± 28.92
IL-10	415.02 ± 7.14
TGF-β	282.83 ± 6.28
IL-6	123.99 ± 3.04

Characteristics of STZ Induced T2DM in HFD rat model

Measuring blood glucose, C-peptide and HOMA-IR, respectively, validated the successful HFD induced hyperglycaemia in T2DM rat model (**Figure 2**). A week following STZ injection, blood glucose levels in the STZ-treated rats were significantly increased compared to untreated rats ($p < 0.001$). The C-peptide secretion was significantly decrease after STZ treatment ($p < 0.001$). There was also a significant increase of HOMA-IR in STZ induced rats compared to untreated rats ($p < 0.001$, respectively).
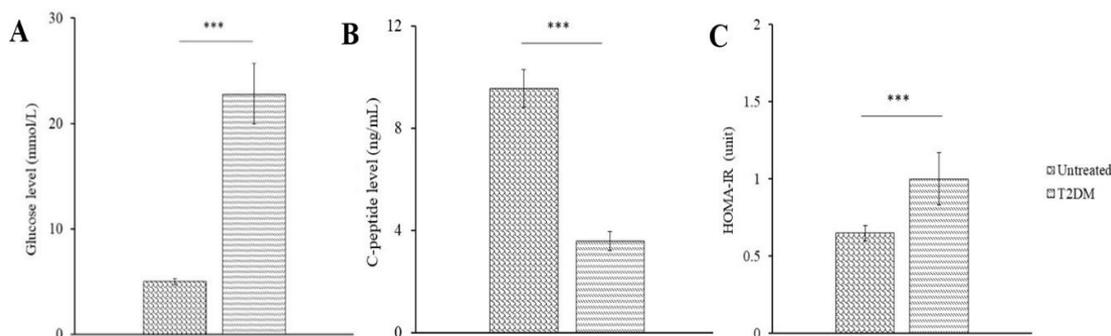

Figure 2 Analysis of A) blood glucose, B) C-peptide, and C) HOMA-IR following HFD-induced hyperglycaemia in rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

SH-MSCs ameliorate hyperglycaemia in HFD-induced T2DM rats

T2DM rats displayed persistent hyperglycaemia, characterized by high level of blood glucose, C-peptide and HOMA-IR. These also followed by a progressive loss in body weight and higher mortality after fasting. Following persistent T2DM in rats, we used SH-MSCs at dose 250 and 500 µL to treat T2DM rats once a week in 3 weeks. The data showed that SH-MSCs at dose 250 and 500 µL significantly decreased the blood glucose level ($p < 0.001$). The HOMA-IR was also significantly decreased following 500 µL SH-MSCs treatment ($p < 0.05$). There was also significant increase of C-peptide level after 250 and 500 µL SH-MSCs treatment in T2DM rats ($p < 0.001$). These results together demonstrate that SH-MSCs injection could contribute to reduce hyperglycaemia in T2DM rats.
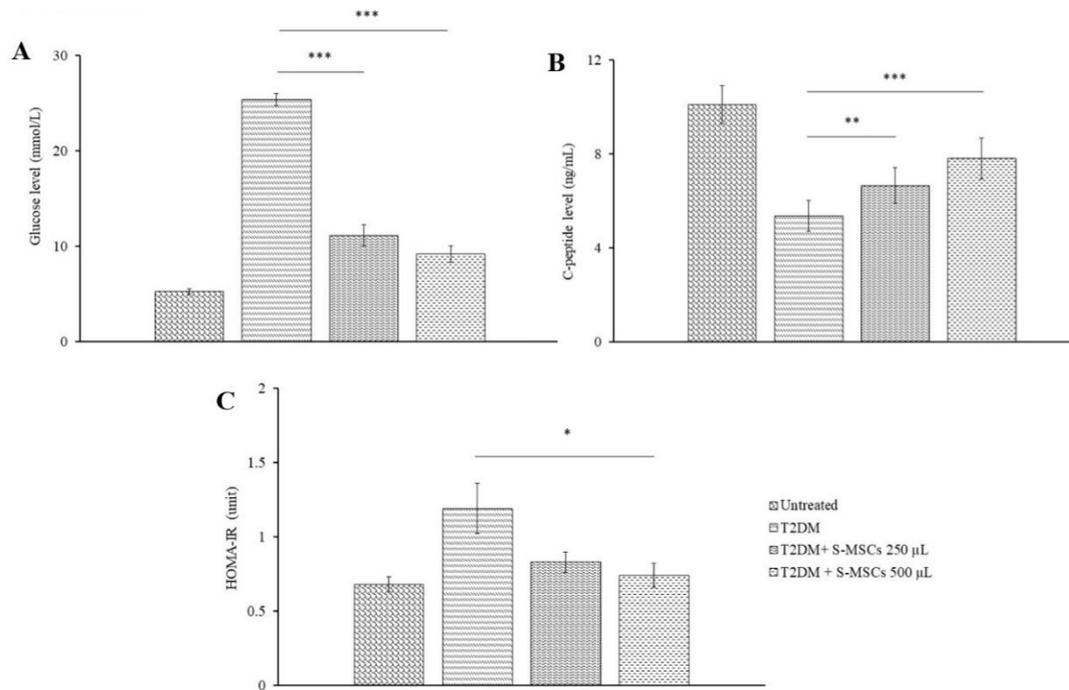
Figure 3 SH-MSCs attenuated changes in A) blood glucose, B) C-peptide and C) HOMA-IR in HFD-induced T2DM rats. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

SH-MSCs favour the histological structure recovery in HFD-induced T2DM rats

Pancreas sections of untreated rats showed normal shape of pancreatic islet (**Figure 4**). However, the HFD-induced T2DM rats showed pathological changes of Langerhans islets which were deranged and shrunken. There was also a decrease of Langerhans islet area after T2DM induction. SH-MSCs at 250 and 500 µL doses restored pancreatic islet architecture. There was a significant increase of Langerhans islet area following 250 µL ($p < 0.05$) and 500 µL SH-MSCs ($p < 0.01$), which 500 µL SH-MSCs provided optimal enhancement.
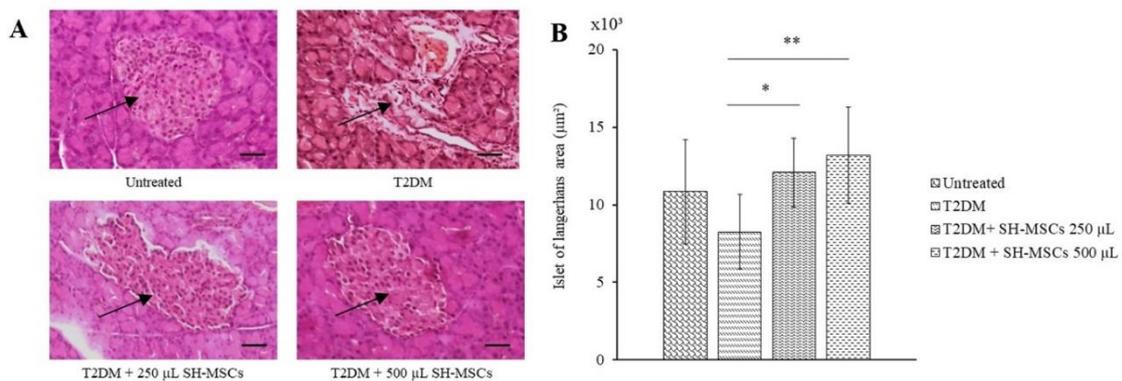

Figure 4 A) Histological appearance of Langerhans islet showed SH-MSCs favour the histological structure recovery of Langerhans islet in HFD-induced T2DM rats. B) A representative diagram of islet of Langerhans area following treatments. Scale bar = 50 µm. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

SH-MSCs inhibit proinflammatory milieu and promote the shift of type 2 macrophage in HFD-induced T2DM rats

We measured the level of IL-6 after 21 days treatment using ELISA (**Figure 5**). HFD-induced T2DM rats showed a high level of IL-6 compared to untreated rats. SH-MSCs at 250 µL inhibited the level of IL-6 in HFD-induced T2DM rats. Moreover, there was also significant and optimal decrease of IL-6 level

following 500 µL SH-MSCs treatment in HFD-induced T2DM rats ($p < 0.001$). On the other side, we analysed the type 2 macrophage polarization through CD163 expression in HFD-induced T2DM pancreatic sections using IHC (**Figure 6**). HFD-induced T2DM did not change the percentage of CD163 positive area. SH-MSCs at both 250 and 500 µL doses significantly induced the percentage of CD163 positive area ($p < 0.001$). These results suggest that SH-MSCs could play a role in inhibiting proinflammatory condition through promoting the type 2 macrophage polarization.
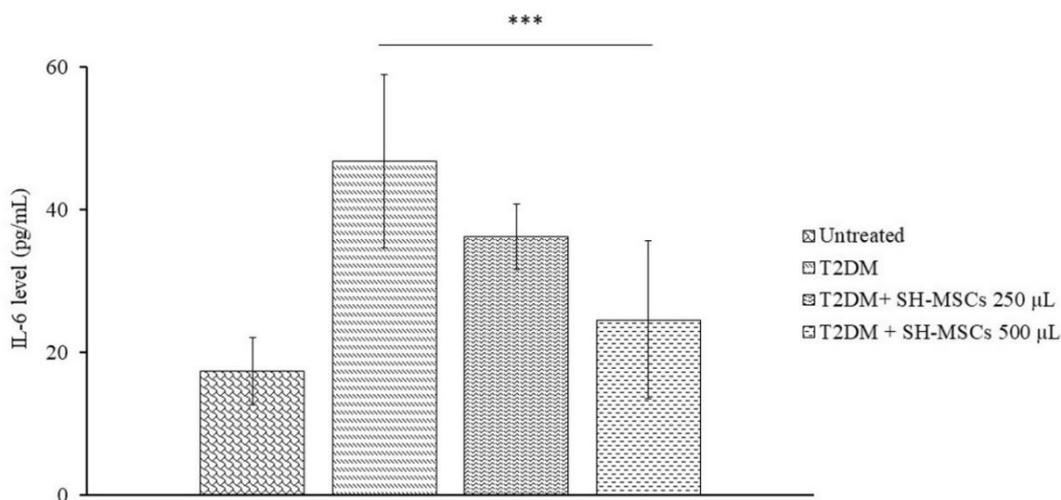

Figure 5 ELISA of the level of IL-6 in HFD-induced T2DM rats following treatments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
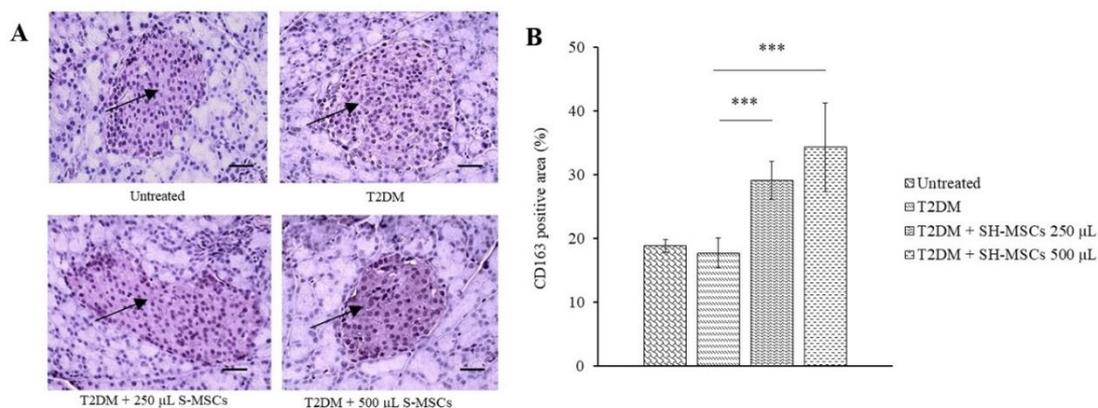

Figure 6 A) IHC appearance showed SH-MSCs increase the percentage of CD163 positive area in HFD-induced T2DM rats. B) A representative diagram of CD163 positive area percentage following treatments. Scale bar = 50 µm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Discussion

Previous studies revealed that MSCs have been shown to be capable of lowering glucose levels in T2DM [12-15]. Based on current studies, the underlying mechanism of MSCs therapeutic impact on hyperglycaemia was thought to include islet regeneration, including direct differentiation into functionally competent b-cells [17,18]. However, contradictory studies have revealed that the limited number of MSC-derived functional β-cells *in vivo* and the small amount of insulin production by these cells appeared to be insufficient to sustain euglycemia. These studies suggested that exploring alternative and potential capacity of soluble molecules contained in MSCs to ameliorate insulin resistance in typical T2DM rats is needed. In this study, we used SH-MSCs isolated from H-MSCs-CM using tangential flow filtration regarding our prior study [16]. Our previous study also reported that hypoxic pre-condition of MSCs could increase the secretome profile 7 - 10 times higher than normoxic condition [19]. We used HFD along with the

administration of STZ to induce T2DM in a rat model. We then performed SH-MSCs infusion once a week in 3 weeks after STZ injection and determined its potency in ameliorating HFD-induced T2DM rats.

In present study, the characterization results of SH-MSCs indicated the successful separation before experimental study. Our results proved that the treatment using SH-MSCs (every week, totalling 3 doses) were found to significantly ameliorates the blood glucose and C-peptide level in HFD-induced T2DM rats. As expected, SH-MSCs at dose 250 µL showed a similar effect to a dose of 500 µL in ameliorating glucose level and improving C-peptide expression (**Figures 3A and 3B**). This further confirms the potential of SH-MSCs as an adjunctive treatment for T2DM. The effective SH-MSCs infusion in the improvement of T2DM indicated that the isolated SH-MSCs carry the component of mediators from H-MSCs that inhibit blood glucose level. According to previous studies, MSCs infusion in T2DM rats reduces IR and hyperglycaemia [12,15]. Therefore, further analysing the insulin resistance in T2DM after SH-MSCs treatment is needed.

In current study, we obtained the fasting serum insulin and fasting blood glucose to compute the HOMA-IR. Previous studies revealed that solely analysing fasting serum insulin could not reflect the actual state of insulin secretion [20]. Some may argue that the fasting serum insulin level may indicate the function of the pancreas response to blood glucose homeostasis. However, this absolute value of fasting serum insulin may not reflect the actual state of insulin secretion during food intake [15]. Previous studies showed that the actual level of insulin production could not be determined just by analysing fasting blood insulin [21,22]. We determined HOMA-IR values and found that there was significant decrease after the treatment of SH-MSCs at dose 500 µL. This finding suggests that the treatment of SH-MSCs using this concentration may be able to regenerate and repair the damaged pancreatic islet in T2DM rats. To strengthen our results, we further conducted a histological analysis to determine the pancreatic cells. Therefore, it is crucial to determine whether or not SH-MSCs may potentially regenerate injured pancreas by pancreatic islet histological evaluation.

Histological evaluation of T2DM rats pancreatic section using HE staining showed pathological alterations of Langerhans islets which were disordered and shrunken. The SH-MSCs at 250 and 500 µL doses showed significant protection and restoration of pancreatic islet morphology compared to untreated T2DM rats. Previous study reported that MSCs and its exosomes could alleviate pancreatic damage and promote islet regeneration in T2DM rat [13-15]. These findings suggest that SH-MSCs through their growth factors could exert a regenerative and protective effect on diabetic rat pancreas.

For further explanation regarding the regeneration of pancreatic cells by SH-MSCs, we evaluated the proinflammatory mediators using ELISA. In this study, we found that there was a trend of inhibition of IL-6 after SH-MSCs treatment at dose 250 µL. Moreover, SH-MSCs at dose 500 µL performed significant reduction level of IL-6 as potential proinflammatory mediators in T2DM rats. These findings demonstrate that SH-MSCs at dose 500 µL robustly inhibit the proinflammatory mediators through plentiful anti-inflammatory molecules, including IL-10. Previous study reported that hypoxic secretom in doses 400uL successfully cure T1DM by decreasing IL-6 levels [15]. Further exploration of anti-inflammatory condition in T2DM rats were examined by analysing type 2 macrophage through CD163 expression in pancreatic cells using IHC. HFD-induced T2DM did not change the percentage of CD163 positive area. After treatments, SH-MSCs at both doses 250 and 500 µL significantly increased the percentage of CD163 positive area in the pancreas of T2DM rats. We suggest that IL-10 contained in SH-MSCs could shift the macrophage polarization from type 1 proinflammatory into type 2 anti-inflammatory. Specifically, IL-10 promote type 2 macrophages polarization through the STAT3 pathway activation [23]. This activation induces the expression of suppressor of cytokine signalling 3 (SOCS-3) leading to the inhibition of NF-kB pathway [24]. These mechanisms promote the polarization of type 1 into type 2 macrophages [25] that play roles in proinflammatory milieu inhibition and pancreatic regeneration [26].

In this study, we did not go further to investigate the detailed mechanism of SH-MSCs to promote the polarization of type 2 macrophages. We did not analyse the expression of CD68 as the potential marker of type 1 proinflammatory macrophage. We also did not analyse the level of other proinflammatory and anti-inflammatory mediators, such as TNF-α, IFN-γ, IL4, IL13 and TGF-β. To fully explore the exact mechanism of macrophage polarization, further study related pathway, such as STAT3, NF-kB and SOCS3 is needed.

Conclusions

Current study represents a novel report on SH-MSCs to ameliorate glucose uptake and insulin resistance in HFD-induced T2DM rats by lowering HOMA-IR and suppressing blood glucose escalation. Histological analysis demonstrated the regenerative and protective effects of SH-MSCs on the morphology of the pancreatic islets. Moreover, serum analysis showed that SH-MSCs treatment could be beneficial for

diabetic rats to inhibit inflammatory condition. The modulation of proinflammatory milieu by SH-MSCs was supported by the increase of CD163 percentage as a firm surface marker of type 2 anti-inflammatory macrophage. Overall, current study findings could present valuable information to develop SH-MSCs as a novel therapy in alleviating T2DM by restoring glucose homeostasis in diabetic patients.

Acknowledgements

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