ELEVATION OF THE ANTI-INFLAMMATORY CYTOKINE INTERLEUKIN-10 IN DIABETIC ERECTILE DYSFUNCTION RAT MODEL THROUGH UMBILICAL CORD MESENCHYMAL STEM CELLS ADMINISTRATION

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Abstract

Diabetic erectile dysfunction (ED) is a common complication of type 2 diabetes mellitus. The pathophysiology of ED is closely associated with the toxic effects of hyperglycemia, which leads to an elevation of reactive oxygen species (ROS) and promotes the secretion of pro-inflammatory cytokines. Prolonged exposure to high glucose levels causes monocytes to become proinflammatory, resulting in the expression and secretion of interleukin-10 (IL-10), which in turn reduces glucose uptake in an autocrine and paracrine manner, transforming monocytes into an anti-inflammatory phenotype. Umbilical cord mesenchymal stem cells (MSCs), known for their immunoregulatory properties, have gained significant interest as a potential strategy for regulating functional cells and tissues, including oxidative stress in ED. This study aims to demonstrate the ability of MSCs to regulate oxidative stress through IL-10 in ED. A total of 20 male Sprague-Dawley rats (6 to 8 weeks old) were randomly divided into four groups: healthy group, negative control group, MSCs $1x10^6$ (T1 group), and MSCs $3x10^6$ (T2 group). After a 16-hour fast, 20 rats were randomly selected and intraperitoneally injected with streptozotocin to induce diabetes mellitus (DM). At 8 weeks after STZ injection, rats with ED were identified based on their unresponsiveness to erectile stimulation within 30 minutes. After MSCs treatment, the rats were sacrificed, and blood samples were prepared for examination. The level of IL-10 in ED showed a significant difference compared to the healthy group. MSCs treatment increased the IL-10 levels in the T1 and T2 groups up to 727.93± 19.09 and 675.54±9.27, respectively. The increase in IL-10 levels was supported by a dose-dependent decrease in blood glucose levels. MSCs have the potential to regulate ED by increasing the levels of the anti-inflammatory cytokine IL-10.

Keywords MSCs, erectile dysfunction**,** IL-10, blood glucose level

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Introduction

Diabetic erectile dysfunction (ED) is a prevalent complication of diabetes mellitus, affecting a significant proportion of diabetic patients [1–3]. It is characterized by impaired penile vascular function and is often attributed to the presence of oxidative stress within the penile tissues. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, plays a crucial role in the pathogenesis of diabetic ED by causing endothelial dysfunction and impairing nitric oxide (NO)-mediated vasodilation [4–7]. Additionally, the increased level of ROS induces inflammation, which is correlated with suppressed levels of anti-inflammatory cytokines, including interleukin-10 (IL-10) [8,9]. Previous study also reported that IL-10 has protective effects against endothelial dysfunction, oxidative stress, and inflammation, all of which contribute to the development and progression of diabetic ED [10]. However, current treatment options for diabetic ED are limited and often provide suboptimal outcomes due to uncontrolled inflammation conditions [11]. Therefore, alternative therapeutic approaches are being explored to improve erectile function in diabetic patients by inhibiting inflammation.

Mesenchymal stem cell (MSCs) therapy has emerged as a promising therapeutic approach for various disorders, including diabetic complications [12,13]. MSCs possess unique regenerative, immunomodulatory, and antiinflammatory properties, making them attractive candidates for the treatment of diabetic ED [14,15]. Several studies have shown that MSCs can improve erectile function and restore penile tissue integrity in animal models of diabetic ED [14]. However, the underlying mechanisms by which MSCs exert their therapeutic effects in diabetic ED, particularly in relation to their anti-inflammatory properties, remain incompletely understood. IL-10 can also prevent the production of nitric oxide induced by IL-1β in β cells, maintaining insulin secretory function even at high glucose levels [16]. Overexpression of IL-10 inhibits IL-1β-induced Fas expression and apoptosis of insulin-secreting cells. Interleukin10 has pleiotropic effects on immunoregulation and inflammation, but it also mediates autoimmune responses and prevents β-cell death by inhibiting caspase-3 activity and attenuating cytokine-induced cytokine Fas expression [17]. Previous studies have hypothesized that high levels of IL-10 prevent the metabolic syndrome of type 2 diabetes by inhibiting pro-inflammatory cytokines such as TNF and IL-6, increasing tyrosine kinase activity of the insulin receptor, and reducing lipolysis [18]. Therefore, IL-10 is considered to protect against type 2 diabetes metabolic syndrome [19]. Interleukin-10 expression significantly increases during the convalescent phase in response to short-term exposure to high glucose [20]. IL-10 is a potent immunosuppressive cytokine that inhibits TLR signaling and the production of pro-inflammatory cytokines through various mechanisms [21]. IL-10 also inhibits glucose uptake in lipopolysaccharide-stimulated macrophages [22]. Long-term exposure to high glucose causes mitochondrial damage and leads to a pro-inflammatory response [23–26]. IL-10 levels were higher in diabetic erectile dysfunction (DED) and decreased significantly a few days after the intervention [14,27,28]. Based on these findings, we aimed to evaluate the antiinflammatory potency of MSC therapy in a diabetic erectile dysfunction rat model.

Methods

Animals and Ethical Clearance

The animal experiments conducted in this study followed the regulations set forth by the Ethical Committee of the Faculty of Medicine, Universitas Sumatera Utara. Approval for the study was granted by Ethical Committee under the number 370/KEP/USU/2020. Male Sprague-Dawley rats, weighing between 200-250g and aged 8 weeks, were obtained from local breeder Semarang, Central Java, Indonesia. Prior to the experiments, the rats underwent a fasting period 16 hours. Out of the 20 rats, 15 were randomly chosen and intraperitoneally administered streptozotocin (STZ, 60 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) [20]. The remaining 5 rats were assigned to the non-diabetic control (NC) group, receiving 500 µL phosphate buffer saline (PBS) instead of the STZ injection. After 3 days of STZ injection, blood glucose levels were measured using a pharmaceutical-grade glucometer (Easy-Touch; Roche, Basel, Switzerland). Among the 20 rats, 15 (75.00%) exhibited fasting glucose levels exceeding 300 mg/dl, indicating the presence of diabetic conditions. At 8 weeks after STZ injection, apomorphine (APO, 100 µg/kg; Sigma-Aldrich, St. Louis, MO, USA) was employed to identify the diabetic rats by administering it through the loose skin on their neck. All the 15 diabetic rats showed unresponsive erectile stimulation within 30 minutes and were confirming the presence of diabetic erectile dysfunction (ED) [15].

Research Design

This research utilized a total of 24 rats. Six healthy rats were assigned to the non-diabetic control (NC) group, while the 18 selected rats with ED were randomly divided into three groups, with six rats in each group: the PC group (ED rats receiving 500 µL PBS), T1 group (ED rats treated with 500 µL PBS containing $1x10^6$ MSCs), and T2 group (ED rats treated with 500 µL PBS containing $3x10^6$ MSCs).

MSCs Isolation and Culture

MSCs were isolated from the umbilical cord (UC) collected from 19-day pregnant female rats and cultured following previously standardized methods. Under aseptic conditions, the UC tissues were cut into smaller pieces and placed in a culture flask containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin/streptomycin, 0.25% amphotericin B, and 10% fetal bovine serum (FBS). The culture flasks were maintained at 37°C in a humid atmosphere with 5% CO2, and the culture medium was replaced every 3 days. When the MSCs reached approximately 80% confluence, they were passage into new culture flasks. MSCs at passage 4 were used for the subsequent experiments [29].

Differentiation of MSCs

To induce osteogenic differentiation, MSCs at passage 4 were cultured in an osteogenic differentiation medium consisting of MesenCult™ MSC Basal Medium (Mouse) supplemented with MesenCult™ Osteogenic Stimulatory Supplement (mouse), L-Glutamine, penicillin/streptomycin, and amphotericin B. The medium was replaced every 3 days, and after 21 days of induction, Alizarin red staining was performed to detect calcium deposition, which appeared as red coloration [30].

IL-10 level analysis

IL-10 levels were quantified through ELISA analysis using the Human-IL-10- E-EL-H0103 kit from Elabscience Company (China), following the manufacturer's instructions [31].

Blood glucose level

Whole blood was collected from tail capillary at weeks 8 were measured using Glucose Stick Easy-Touch (Roche, Basel, Switzerland) under manufacturer's instructions.

Statistical analysis

The data are presented as means ± standard deviation (SD). Statistical calculations were conducted using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). The statistical significance of group differences was evaluated using the Kruskal-Wallis test, followed by Mann-Whitney postdoc analysis. A p-value of less than 0.05 and 0.001 was considered statistically significant.

Results

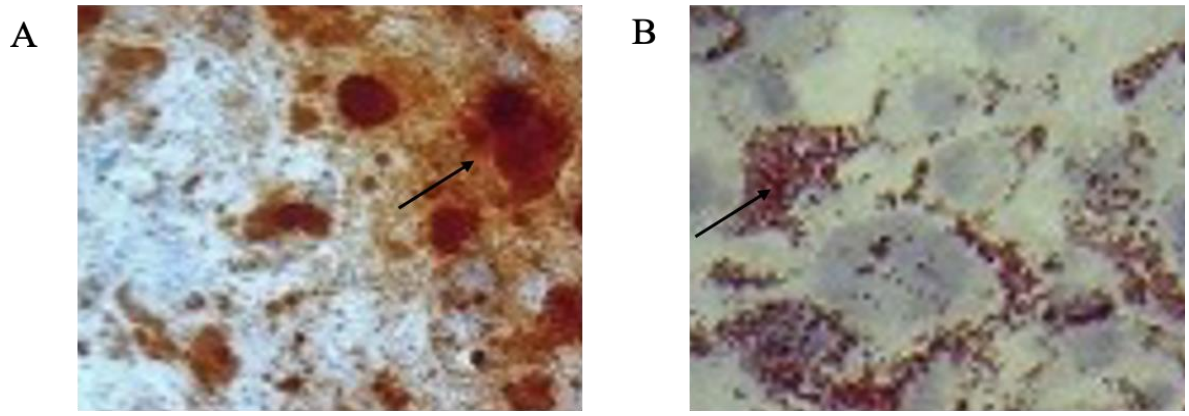

Figure 1. Illustrates the MSCs attributes. (A) The outcome of the osteogenic differentiation examination demonstrates the presence of red-colored calcium deposits, indicating successful post-culturing with an osteogenic differentiation medium. (B) The adipogenic differentiation test reveals the detection of fat deposits on the MSCs, visually highlighted in red through Oil Red O staining at a magnification of 400x.

After reaching the fourth passage, the MSCs in culture exhibited a spindle-like cell morphology and adhered to the flask's bottom when observed under a microscope. Validating the MSCs, their osteogenic and adipogenic capabilities were assessed. Osteogenic differentiation was confirmed through the presence of red-stained calcium deposits using Alizarin red staining (Figure 1A), while adipogenic differentiation was induced using a specific adipogenic medium, resulting in the formation of adipogenic capacity (Figure 1B).

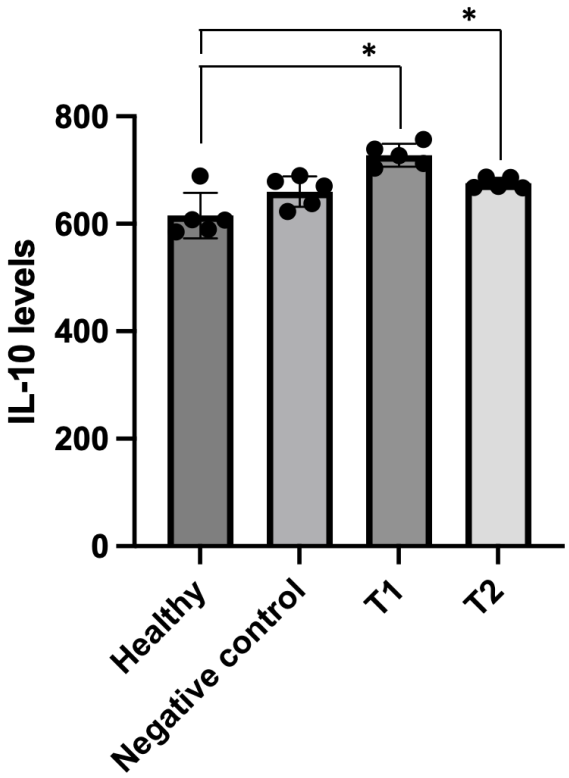

Figure 2. MSCs increased the IL-10 level in Diabetic Erectile Dysfunction Rats model. Values are mean ± SD, *p < 0.05 vs healthy (n=5).

In the Diabetic Erectile Dysfunction Rats model, treatment with MSCs T1 and T2 led to an significantly increase in IL-10 levels up to 727.93± 19.09 and 675.54±9.27, respectively; however, this increase was not found to be dosedependent (Figure 2).

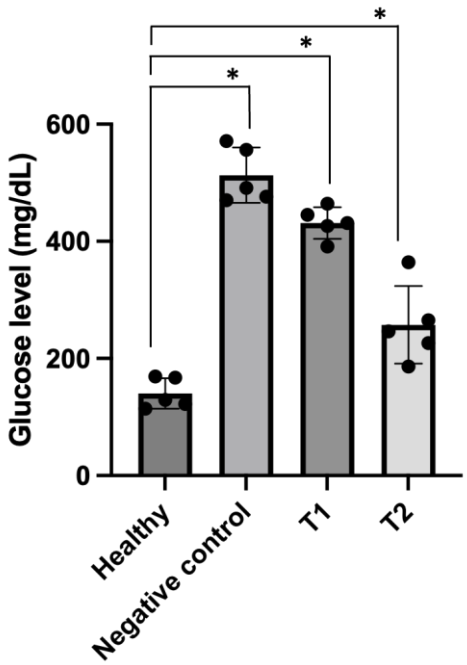

Figure 3. MSCs decreased glucose level in Diabetic Erectile Dysfunction Rats model. Values are mean ± SD, *p < 0.05 vs healthy (n=5).

The presence of MSCs significantly decreased glucose level in doses-dependent manner, the treatment of T1 significantly suppressed blood glucose level up to 431.40± 27 mg/dL, interestingly the treatment of T2 decreased blood glucose level 2-fold than T1 (Figure 3).

Discussion

The findings of this study demonstrate the potential of mesenchymal stem cells (MSCs) to induce interleukin10 (IL-10) levels in diabetic erectile dysfunction (ED) by exerting their anti-oxidative activity. Diabetic ED is a common complication of diabetes mellitus characterized by impaired penile vascular function, often attributed to oxidative stress within penile tissues [11]. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, plays a crucial role in the pathogenesis of diabetic ED by causing endothelial dysfunction and impairing nitric oxide (NO)-mediated vasodilation [4,10].

In this study, MSCs therapy was explored as a potential therapeutic approach for diabetic ED, given the unique regenerative, immunomodulatory, and anti-inflammatory properties of MSCs [32–34]. Previous studies have shown that MSCs can improve erectile function and restore penile tissue integrity in animal models of diabetic ED [14,15]. However, the specific mechanisms underlying the therapeutic effects of MSCs, particularly in relation to their anti-oxidative activity and modulation of IL-10 levels, remain incompletely understood.

The results of our study indicate that MSC therapy leads to an increase in IL-10 levels in the diabetic ED rat model. IL-10 is a cytokine known for its anti-inflammatory and immunomodulatory effects [18,35,36]. It has been shown to have protective effects against endothelial dysfunction, oxidative stress, and inflammation, all of which contribute to the development and progression of diabetic ED. IL-10 can inhibit the development of fibrosis by competitively binding to the TGF receptor resulting in decreased TGF-β expression. Interaction of IL-10 to the TGF-β receptor induces serine/threonine kinase activity leading to the induction of SMAD2 or SMAD3 downstream signaling proteins leading to activating insulin activity [20]. The upregulation of IL-10 observed in our study suggests that MSCs may exert their therapeutic effects in diabetic ED through the induction of anti-inflammatory and anti-oxidative pathways.

The anti-oxidative activity of MSCs is of particular significance in the context of diabetic ED, as oxidative stress is a major contributor to endothelial dysfunction and impaired vasodilation in the penile tissues [6]. By reducing ROS production and enhancing antioxidant defense mechanisms, MSCs may help restore the balance and alleviate oxidative stress-induced damage in the penile tissues, leading to improved erectile function.

The findings of our study provide further insights into the potential mechanisms underlying the therapeutic effects of MSCs in diabetic ED. By inducing IL-10 levels and exerting their anti-oxidative activity, MSCs may contribute to the restoration of penile vascular function and improvement of erectile function in diabetic patients. These findings support the exploration of MSC therapy as a promising approach for the treatment of diabetic ED, offering a potential alternative to current limited treatment options [15].

However, it is important to acknowledge the limitations of this study. Further research is needed to elucidate the precise mechanisms by which MSCs modulate IL-10 levels and exert their anti-oxidative activity in the context of diabetic ED. Additionally, clinical studies are required to validate the findings in animal models and evaluate the safety and efficacy of MSC therapy in human subjects.

In conclusion, our study highlights the potential of MSC therapy to induce IL-10 levels and mitigate oxidative stress in diabetic ED. These findings contribute to our understanding of the therapeutic mechanisms underlying MSC therapy and provide a basis for future research and clinical investigations in the field of diabetic ED treatment.

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Authors declares no conflict of interest.

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