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Human Wharton's Jelly Mesenchymal Stem Cells Improve Insulin Levels and Reduce Weight Gain in Aging Female Rats

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ABSTRACT

Background: This study aimed to analyze the effect of repeated intravenous injections of human Wharton's jelly mesenchymal stem cells (hWJ-MSCs), administered at a dose of 1 × 10^6 cells/kg four times at 3-month intervals, on insulin levels and weight gain in aging female rats.

Materials and Methods: Twelve female rats were divided into three groups: Group A (3-month-old young females, untreated control), Group B (24-month-old aged females injected with 0.4 mL of 0.9% NaCl as vehicle control), and Group C (24-month-old aged females injected with 1 × 10^6 cells/kg of hWJ-MSCs suspended in 0.4 mL of 0.9% NaCl). Injections were administered at baseline and then every 3 months for a total of four doses. Twelve months after the first injection, the rats were anesthetized and sacrificed. Serum insulin levels were measured using ELISA, and immunohistochemical staining was performed to detect hWJ-MSCs homing to pancreatic tissue.

Results: Rats treated with hWJ-MSCs showed a trend toward lower body weight compared to the aged vehicle-control group. Insulin levels in treated aged rats were significantly higher than those in the aged control group ($p < 0.05$). Immunohistochemical analysis revealed that hWJ-MSCs were extensively distributed within the islets of Langerhans of treated aged rats.

Conclusion: Repeated intravenous administration of hWJ-MSCs (1 × 10^6 cells/kg, four doses over 12 months) led to successful migration and homing to the islets of Langerhans in aging female rats. Treated animals exhibited significantly increased insulin levels and a trend toward reduced weight gain, suggesting a potential metabolic benefit of hWJ-MSC therapy in physiological aging.

Keywords: Aging; Female; Insulin; Weight; Stem cell

INTRODUCTION

Aging in females is associated with weight gain and insulin resistance. The basal metabolic rate in females decreases with increasing age, meaning that the body requires less energy to perform its functions. Consequently, unused calories are stored as lipids[1]. This weight gain is also influenced by decreased physical activity, which reduces calorie burning and can lead to weight gain[2]. Another contributing factor is sarcopenia—age-related changes in body composition—in which muscle mass decreases while lipid mass increases. Because muscle tissue burns more calories than lipid tissue, a decline in muscle mass reduces the daily calories needed and causes weight gain[3].

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On the other hand, in aging females who have undergone menopause, the risk of insulin resistance will increase because of decreasing estrogen hormone levels and increasing visceral lipids[4]. Decreasing estrogen hormone levels reduce insulin sensitivity, causing the body to require more insulin to control blood sugar levels. This condition increases pancreatic workload and, ultimately, the risk of insulin resistance[5]. Estrogen is also an important factor in adipose tissue distribution; therefore, decreasing estrogen levels increase visceral lipid accumulation and promote metabolic syndrome. Consequently, inflammation associated with visceral lipids increases as well[6].

Previous studies have shown that mesenchymal stem cell injection improves ovarian function by increasing follicle counts and anti-Müllerian hormone (AMH) levels, and restores fertility in mice[7]. Human umbilical cord mesenchymal stem cell (hUC-MSC) injection has also been reported to repair ovarian function by reducing apoptosis and increasing ovarian weight and follicle counts in rats[8]. Similarly, human placenta-derived mesenchymal stem cells (hPD-MSCs) have demonstrated beneficial effects in ovarian aging rats, including reduced body weight and improved insulin metabolism[9].

Other studies have reported that human Wharton's jelly mesenchymal stem cell (hWJ-MSCs) exhibit superior characteristics compared with other MSC sources, including stronger immunomodulatory and higher immunosuppressive ability. hWJ-MSCs also show a higher growth rate than bone marrow mesenchymal stem cells (BM-MSCs), adipose tissue and placenta. In addition, hWJ-MSCs demonstrate higher osteogenetic ability than BM-MSCs and placenta. The expression levels of MHC II, TLR24, TLR3, JAGI, NOTCH2 and NOTCH3 are lowest in hWJ-MSCs[10].

hWJ-MSC transplantation in aging female rats can repair pancreatic function by increasing the diameter of pancreatic islets, the total number of pancreatic islets, and the population of pancreatic islet cells[11]. hUC-MSC injection has also shown therapeutic effects in repairing traumatic pancreatitis by decreasing histopathological scores[12]. Another finding proves that mesenchymal stem cell therapy can restore β cells and control hyperglycemia in streptozotocin-induced diabetic rats[13]. Therefore, this study aimed to investigate the effect of different doses of hWJ-MSC injection on insulin hormone levels and weight gain in physiologically aging female rats.

MATERIALS AND METHODS

This research was approved and licensed by the ACUC Animal Care and Use Committee (ACUC) of the Veterinary Hospital of Bogor Agricultural University, Indonesia (Approval No. 21-2016 ACUC RSHP FKH-IPB).

Experiment Animal

In this study, 12 rats were used, consisting of young female rats (three months old and weighing 96–112 grams) and aged female rats (24 months old and weighing 210–291 grams). One month of rat age is approximately equivalent to 2.5 human years. According to a previous report, three months of rat age is approximately equivalent to 7.5 human years, and 24 months of rat age is approximately equivalent to 60 human years[14]. The rat strain was Sprague Dawley (SD), and the rats were divided into three groups:

Group A (Control): Three-month-old female rats with no treatment.

Group B (Control): Twenty-four-month-old aged female rats injected with 0.4 mL of NaCl (0.9%) through the tail vein. The injections were administered four times at three-month intervals.

Group C (Treatment): Twenty-four-month-old aged female rats injected with hWJ-MSCs at a dose of 1×10^6 cells/kg through the tail vein. The injections were administered four times at three-month intervals.

According to previous studies, four intravenous injections of hWJ-MSCs yield better results than two injections[15].

Procedure

This study was conducted over a 12-month period. Food and water were provided ad libitum. The animals were maintained under a 12-hour light/dark cycle. Rat weights were monitored weekly using a digital scale and recorded in grams. The rats were sacrificed at the end of the experiment.

HWJ-MSCs Isolation

HWJ-MSCs were obtained from the Stem Cell and Cancer Institute. According to the isolation procedure of the Stem Cell and Cancer Institute, fresh human umbilical cords (UCs) were collected from women aged 25-40 years after normal vaginal delivery. The UCs were cut longitudinally into 1-2 mm segments and plated onto tissue culture plastic. The tissues were then cultured in MEM-α supplemented with 2 mM GlutaMax, 20% FBS and penicillin-streptomycin-amphotericin B. Cultures were incubated in a humidified atmosphere containing 5% $C0_2$ at 37° C for 3 weeks. The adherent cells and tissue fragments were detached using trypLE-EDTA solution and subsequently washed with basal medium to remove the trypLE-EDTA. The harvested cells were replated at a density of 8 x 103 cells/cm2. HWJ-MSCs were cultured under normoxic conditions (95% air [21% O2]/5% CO2) and hypoxic conditions (5% and 2.5% O2). Flow cytometric analysis showed that hWJ-MSCs were positive for CD105 and CD73 and negative for CD14, CD19, CD34, CD45 and HLA-II[16].

Measurement of Insulin Hormone Levels

Insulin levels were measured from blood samples using an enzyme-linked immunosorbent assay (ELISA) with a rat insulin ELISA kit (96-well).

Immunohistochemistry

In this study, mouse monoclonal antibodies specific for a human marker (anti-mitochondrial antibody) were used to assess the homing of hWJ-MSCs in pancreatic tissue. Five fields of view were examined per slide. Slides were documented and examined using an Olympus CX31 light microscope. Cells were observed using ImageJ software. The results were analyzed descriptively.

Statistical analysis

Body weight gain and insulin level data were analyzed using the SPSS statistical package (version 23) with nonparametric independent-sample Kruskal–Wallis tests, followed by post hoc tests. Immunohistochemistry observations were analyzed descriptively.

RESULT

Weight Gain

The average body weight of both the control and treated rat groups is presented in Table 1. The data showed a significant difference in average body weight between young and aging rats (control and treated) ($p < 0.05$). Although there was no significant difference in average body weight between aging control rats and aging rats treated with hWJ-MSCs (1×10^6 cells/kg), the data showed that the mean body weight of the hWJ-MSC–treated group was 267 g, compared with 283 g in the control group. These findings indicate a trend toward reduced weight gain in the hWJ-MSC–treated group. Data are presented as mean and standard deviation. Different letters in the same column indicate significant differences at $p < 0.05$ (post hoc test).

Insulin Hormone Level

The average insulin levels in the control and treated groups are presented in Table 2. There was a significant difference in average insulin levels between young rats and aging control rats ($p = 0.013$). However, there was no significant difference in average insulin levels between young rats and aging rats treated with hWJ-MSCs (1×10^6 cells/kg) ($p = 0.433$). The insulin level in treated aging rats (10,494 mIU/L) tended to closely resemble that of the young rat group (11,364 mIU/L). In addition, the insulin level in treated aging rats was significantly different from that in aging control rats ($p = 0.036$), which exhibited an insulin level of 6.609 mIU/L. Data are presented as mean and standard deviation. Different letters in the same column indicate significant differences at $p < 0.05$ (post hoc test).

Table 1: Independent-sample Kruskal-Walli's result of average body weight among young rats, aging control rats, and aging rat treated with hWJ-MSCs (1x 10^6 cells/kg)

Group	Mean (gram)	Standard Deviation	*p*-value
Young Female Rat (Control)[(a)]	105,3	8,3	0,031[a, c]
Aging Female Rat (Control)[(b)]	283,31	26,40	0,17[a, b]
Aging Female Rat (hWJ-MSCs 1x 10^6 cells/kg) [(c)]	267,00	30,43	0,56[b, c]

Table 2: Independent-sample Kruskal-Walli's result of average insulin hormone level among young rats, aging control rats, and aging rats treated with hWJ-MSCs (1x 10^6 cells/kg)

Group	Mean (mIU/L)	Standard Deviation	*p*-value
Young Female Rat (Control) [(a)]	11364	710,0	0,433[a, c]
Aging Female Rat (Control)[(b)]	6609	1757,7	0,013[a, b]
Aging Female Rat (*hWJ-MSCs 1x 10^6* cells/kg*) [(c)]*	10494	1538,6	0,036[b, c]

Immunohistochemistry

Immunohistochemical results are shown in Figure 1. The black arrow in panel A indicates the absence of brown staining (negative), whereas the black arrow in panel B shows a dense brown precipitate, indicating strong positive staining with approximately 70% positive cells. hWJ-MSCs were widely distributed in the islets of Langerhans in treated aging rats. This distribution was associated with significantly increased insulin levels and decreased body weight in physiologically aging female rats. Based on these findings, it can be concluded that hWJ-MSCs are able to home to pancreatic tissue, significantly increase insulin levels, and show a trend toward decreased body weight in physiologically aging female rats.

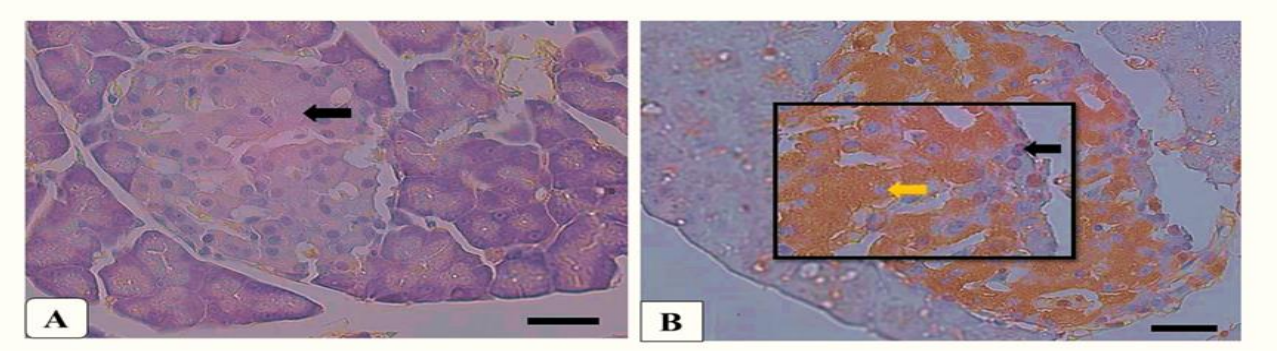

Figure 1**.** Results of immumohistochemistry staining of pancreatic tissue in aging female rats. A = aging control rat: black arrow indicates no brown staining (negative). B = aging rat treated with hWJ-MSCs (1x 10^6 cells/kg): yellow arrow indicates dense brown straining, representing strong positive with bar = 50 µm.

DISCUSSION

Diabetes is associated with decreased pancreatic function and reduced β-cell mass[17]. One promising therapeutic approach for diabetes is stem cell therapy, especially using mesenchymal stem cells. Human Wharton's jelly mesenchymal stem cells have been shown to reduce hyperglycemia, enhance β-cell function, and decrease damage to pancreatic islets in diabetic models induced by streptozotocin treatment[18]. Other studies have also shown that hWJ-MSC injection in physiologically aging female rats (without streptozotocin treatment) can repair pancreatic islet structure[11]. Extensive research has investigated the effects of human Wharton's jelly mesenchymal stem cells on pancreatic function in diabetic models. However, information regarding the effects of hWJ-MSC injection in physiologically aging female rats without streptozotocin treatment remains limited.

Insulin secretion in female Sprague-Dawley rats declines with age, whereas body weight increases with age[19]. This finding is consistent with the results of this research, in which young rats (three months of age) exhibited higher insulin levels (11,364 mIU/L), while aging control rats (24 months of age) showed a significant decrease in insulin levels (6,609 mIU/L). Conversely, the body weight of aging control rats (24 months of age) was higher (283.31 grams) than that of young rats (three months of age; 105.3 grams). Notably, after four injections of hWJ-MSCs at a dose of 1×10^6 cells/kg, insulin levels in aging rats (24 months of age) increased significantly to 10,494 mIU/L, and body weight decreased to 267 grams compared with the aging control group. The small sample size in this study was influenced by the high mortality rate of rats in the control group. This finding is consistent with previous research showing that hWJ-MSCs reduced the mortality rate from 45% in the control group to 10% in the treated group[20].

In this study, hWJ-MSCs in the pancreas of aging rats were detected using monoclonal antibodies (anti-mitochondrial antibodies) visualized with diaminobenzidine (DAB), which produced a brown precipitate. Immunoreactivity in pancreatic tissue after the administration of hWJ-MSCs produced brown precipitate formation in the cytoplasm, while counterstaining with hematoxylin produced a purple coloration in the cell nuclei. In control aging female rats (Figure 1), no brown staining was observed in the cytoplasm of the islets of Langerhans, indicating the absence of hWJ-MSCs. In contrast, aging rats receiving four injections of hWJ-MSCs at a dose of 1×10^6 cells/kg exhibited dense brown precipitates in the cytoplasm of the islets of Langerhans. These findings indicate that hWJ-MSCs successfully migrated and homed to the pancreas, particularly to the islets of Langerhans, in physiologically aging female rats. This result is consistent with previous studies reporting that MSCs can migrate and home to pancreatic tissue within 4 weeks post-injection in diabetic rat models and successfully increase the number of pancreatic beta cells[21].

The migration and homing of hWJ-MSCs in pancreatic tissue can be explained based on previous studies. In principle, mesenchymal stem cells are able to migrate and home to damaged tissues and organs[22]. They can reach pancreatic tissue through the circulatory system[18]. This process occurs when pancreatic injury induces the release of chemokines that interact with hWJ-MSCs. The interaction begins with the binding between chemokine receptors expressed by hWJ-MSCs (CXCR4, CX3CR1, CXCR6, CCR1, and CCR7) and chemokines released by pancreatic cells, including CXCL12, CX3CL1, CXCL16, CCL19, and CCL32[3]. Cytokines and growth factors produced by MSCs promote paracrine mechanisms, which are key contributors to pancreatic regeneration. These factors also play an important role in preventing apoptosis in injured β-cells[24]. In addition, MSCs can induce β-cell regeneration and inhibit T-cell–mediated immune responses[25].

CONCLUSION

The results of this study indicate that body weight and insulin levels in physiologically aging female rats change with age. Four intravenous injections of Wharton's jelly mesenchymal stem cells at a dose of 1×10^6 cells/kg in physiologically aging female rats successfully migrated and homed to the islets of Langerhans, resulted in a trend toward reduced weight gain, and significantly increased insulin levels.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Funding Statement

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Ethics Approval and Consent to participate

This research was approved and licensed by the Animal Care and Use Committee (ACUC) of the Veterinary Hospital of Bogor Agricultural University, Indonesia (Approval No. 21-2016 ACUC RSHP FKH-IPB).

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