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The Effect of Hypoxic Mesenchymal Stem Cell Secretome on TNF-A and MCP-1 Gene Expression

Ihdina Hanifa Hasanal Ibrahim[1*], Agung Putra[2], Titiek Sumarawati[3]

[1] Postgraduate student of Biomedical Sciences, Faculty of Medicine, Universitas Islam Sultan Agung, Jl Kaligawe KM 4 Semarang 50012

[2,3] Department of Biomedical Sciences, Faculty of Medicine, Universitas Islam Sultan Agung, Jl Kaligawe KM 4 Semarang 50012

ABSTRACT: Diabetes mellitus (DM) type 2 is a metabolic disorder with the occurrence of insulin receptor resistance and reduced ability of pancreatic β-cells to secrete insulin, there will be an increase in adipose tissue which causes hypertrophy and hyperplasia. This situation triggers inflammation involving the role of pro-inflammatory cytokines and chemokines such as TNF-α (Tumor Necrosis Factor-Alpha) and MCP-1 (Monocyte Chemotactic Protein-1). Cell-based therapies such as the administration of hypoxic mesenchymal stem cell secretom offer a new paradigm in the management of T2DM. This study aims to determine the effect of hypoxic mesenchymal stem cell (SSPM) administration on TNF-α and MCP-1 gene expression in STZ-induced obese DM rats. This research is experimental with a post-test-only control group design approach. The subjects of the study were 20 male Wistar rats which were randomly divided into 4 groups. Group K1 was given standard feed and distilled water. Group K2 subjects with obesity mellitus type 2 with 0.9% NaCl. Groups K3 and K4, subjects with type 2 obesity mellitus, were treated with secretom doses of 250 µl and 500 µl. The study was conducted at the Sultan Agung Stem Cell Research Institute Laboratory for 21 days by taking blood for examination of TNF-α and MCP-1 gene expression using the PCR method. The result showed that the lowest average TNF expression was in the K4 group (3.19 pg/ml) and the lowest mean MCP-1 expression was in the K4 group (1.57 %). One-way ANOVA test showed a significant difference in TNF- expression with a value of p=0.006 and MCP1 expression showed a significant difference p=0.000 (p<0.05). The Administration of hypoxic mesenchymal stem cell (SSPM) secretom significantly reduced TNF-α and MCP-1 gene expression in STZ-induced obese DM rats.

KEYWORDS: Diabetes mellitus, TNF-α, MCP-1, SPPM

I. INTRODUCTION

Type 2 diabetes mellitus (DM) is a long-term disease characterized by high blood sugar levels due to the body's inability to produce insulin.[1] Metabolic disorders with the occurrence of insulin receptor resistance and reduced ability of pancreatic β-cells to secrete insulin, there will be an increase in adipose tissue which causes hypertrophy and hyperplasia.[2] This situation triggers inflammation involving the role of pro-inflammatory cytokines and chemokines such as TNF-α (Tumor Necrosis Factor-Alpha) and MCP-1 (Monocyte Chemotactic Protein-1).[3] Pharmacotherapy and insulin injections for T2DM patients currently only reduce glycemic levels but cannot repair the pancreatic β-cell damage that occurs. Cell-based therapies such as the administration of hypoxic mesenchymal stem cell secretome offer a new paradigm in the management of T2DM.[4] Hypoxic mesenchymal stem cell (SSPM) secretome has benefits as immunosuppressors and immunomodulator which can improve the function of pancreatic β cells, there is still little research on hypoxic mesenchymal stem cell secretome on TNF-α and MCP-1 gene expression in type 2 DM obesity.[5] According to data International Diabetes Federation (IDF) in 2021 the prevalence of DM sufferers in the 536.6 million or 10.5% living with DM is expected to increase by 783.2 million or 12.2% of the world's population in 2045.[6] Meanwhile, based on IDF 2021 data, there has been an increase in diabetes sufferers in Indonesia where Indonesia ranks 5th in the world and 3rd in Southeast Asia with sufferers of 11.3% of the total age 20 to 79 years.[6,7] The Indonesian Basic Health Research which was carried out in 2018 collected data on DM sufferers in residents aged more than 15 years based on blood sugar examinations and found an increase in the prevalence of DM by 8.5% or around 20.4 million compared to 2013 which was 6. 9%. The biggest increase in prevalence was in DKI Jakarta province, which was 3.4%.[7,8] The World Health Organization predicts an increase in the number of Type 2 DM

patients in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030 and this is predicted to continue to increase in the coming years.[8]

Mesenchymal stem cell secretome (SSPM) obtained from adipose tissue has a greater density compared to bone marrow.[9] These cells are one of the potential and effective candidates in modulating the inflammatory environment and immune cells because SSPM is capable of producing anti-fibrotic, immunomodulating, and anti-apoptotic factors.[5,10] Several studies have shown that SSPM at a dose of 40% from type 2 DM rats has a unique secretion with different angiogenic properties in reducing inflammation and improving pancreatic function.[11]

An imbalance between energy intake and energy used in physical activity will increase fat tissue deposits, resulting in obesity and accumulation of visceral fat.[20] The accumulation of visceral fat results in adipose hypertrophy and causes hypoxia in the endoplasmic reticulum of cells, adipocyte death, and MCP-1 infiltration. If this happens continuously it will increase the release of proinflammatory cytokines from MCP-1 such as TNF-α which will eventually result in local and systemic inflammation which will interfere with the process of insulin production.[12,13]. Currently, the treatment of T2DM patients is more about lifestyle changes and the administration of oral anti-hyperglycemic drugs and insulin for the rest of their lives. This therapy only lowers glycemic levels but cannot completely reduce the causative factors of insulin resistance and does not repair pancreatic β-cell damage that occurs. Therefore, a better therapeutic approach for DMT2 that can increase insulin sensitivity and improve β-cell function will bring benefits to DMT2 sufferers. Research related to the ability to apply hypoxic SSPM in the process of repairing inflammation through MCP-1 and TNF-α to restore insulin sensitivity and repair pancreatic β cells in DMT2 is still not widely carried out. the TNF-α gene and MCP-1 in induced obese mice Streptozotocin (STZ).

II. MATERIAL AND METHOD

This research is an in vitro experimental research method using Post-Test Only Control Group Design. It is a research design where the research results are observed after the treatment is complete. The research subject used male Wistar rats and was divided into four groups, the healthy control group which was standard-fed and given distilled water (K1), the group of subjects with obesity mellitus type 2 with 0.9% NaCL (K2), the group of subjects with obesity mellitus type 2 treated with a dose of 250 µl secretome (K3), and the group of subjects with obesity mellitus type 2 was treated with a dose of 500 µl secretome (K4). The Hypoxic Mesenchymal Stem Cell Secretome used in the study contains IL-10, VEGF, and PDGF factors obtained from the co-culture of SPM Umbilical Cord secretions.

III. RESULT

In this study, the researchers found that the effect of hypoxic mesenchymal stem cell secretome administration on TNF-α and MCP-1 expression in STZ-induced obese rats in male rats of the Wistar strain depended on the dose given (Table 1).

Table 1. Results of Average Analysis, Normality Test, Homogeneity Test on TNF-α and MCP-1 Expression

Variable	Group				
	K1 N=5	K2 N=5	K3 N=5	K4 N=5	Sig.(p)
TNF-α expression					
Mean	1.00	8.00	4.85	3.19	
Std. deviation	0.00	4.63	1.66	3.19	
Shapiro Wilk		0.644*	0.379*	0.418*	
Levene Test					0.003
One Way Anova					0.006***
Up to IL-6 (ng/L)					
Mean	1.00	7.04	2.65	1.57	
Std. deviation	0.00	5.86	1.73	1.36	
Shapiro Wilk	0.154 *	0.424*	0.379*	0.418*	
Levene Test					0.034
One Way Anova					0.000***
Information: * Normal p>0.05 **Homogeneous p>0.05 ***Significant p<0.05					

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Table 1 shows that the highest mean expression of TNF-α was in the second treatment group (K2) in STZ-induced obese rats without administration of hypoxic mesenchymal stem cell secretome. The control group (K1) with standard feed and distilled water showed the lowest average TNF- expression, followed by the fourth treatment group (K4) with hypoxic mesenchymal stem cell secretome administration with a dose of 500 µl and the third treatment group (K3) with cell secretome administration. hypoxic mesenchymal stem at a dose of 250 µl in STZ-induced obese rats. TNF-α expression data based on the Shapiro-Wilk test showed a normal distribution with a p-value <0.05).

The Differences in TNF-α Between Groups

Table 2. Differences in TNF-α expression between the two group

Group	*p-Value*
K1 vs K2	0.156
K1 vs K3	0.039*
K1 vs K4	0.423
K2 vs K3	0.761
K2 vs K4	0.405
K3 vs K4	0.764

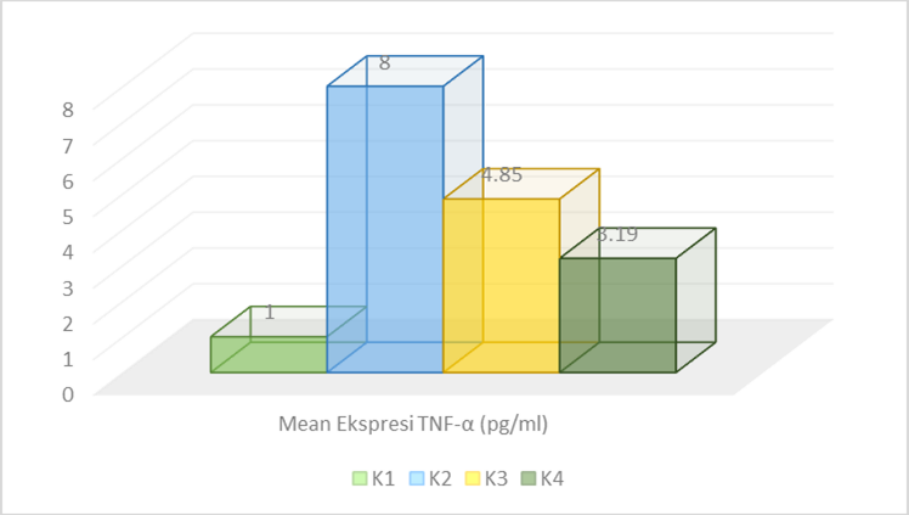

Figure 1. The average of TNF-α expression between groups

The results of the Tamhane test in Table 2 and Figure 1 show that the expression of TNF-α in group K1 did not differ significantly from group K2 with a p-value of 0.156 (p>0.05) and group K4 with a p-value of 0.423 (p>0.05) but there was a significant difference to the K3 group with a p-value of 0.039 (p0.05). Based on the data above, it can be concluded that the administration of hypoxic mesenchymal stem cell secretome at doses of 250 µl and 500 µl had a significant effect on TNF-α expression in STZ-induced obese rats so the hypothesis statement was accepted.

The administration of MCP-1 Expression Between Groups

Table 1 shows that the highest mean expression of MCP-1 was in the second treatment group (K2) in STZ-induced obese rats without administration of hypoxic mesenchymal stem cell secretome. The control group (K1) with standard feed and distilled water showed the lowest average expression of MCP-1, followed by the fourth treatment group (K4) with hypoxic mesenchymal stem cell secretome administration with a dose of 500 µl and the third treatment group (K3) with secretome administration hypoxic mesenchymal stem cells at a dose of 250 µl in STZ-induced obese male Wistar rats. TNF-α expression data based on the Shapiro Wilk test showed a normal distribution with a p-value <0.05 and the homogeneity test using the Levene test was not homogeneous with a p-value <0.05, so data analysis used the parametric test One-Way ANOVA. The results of the One-Way ANOVA test showed significant differences in all groups with a p-value of 0.000 (p <0.05).

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The Differences in IL-6 levels between the two group

Table 3. The Differences in IL-6 levels between the two group

Group	*p-Value*
K1 vs K2	0.075
K1 vs K3	0.176
K1 vs K4	0.382
K2 vs K3	0.134
K2 vs K4	0.103
K3 vs K4	0.935

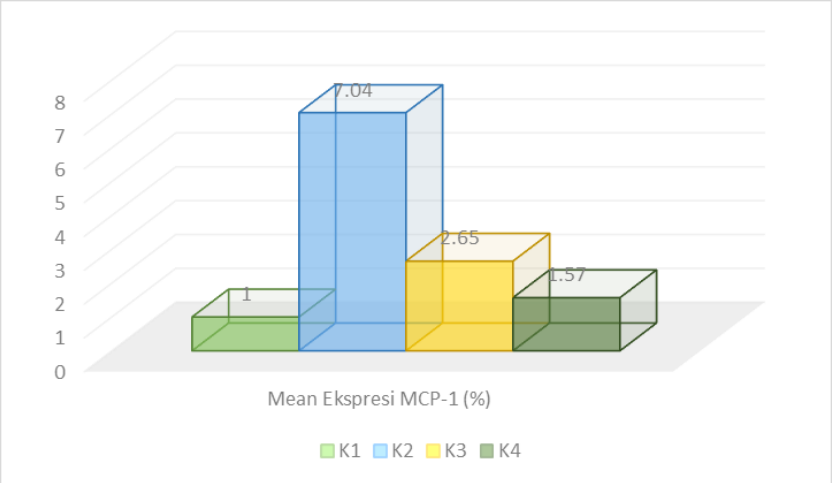

Figure 2. The average of MCP-1 expression between groups

The Tamhane test result in Table 3 and Figure 2 shows that the expression of MCP1 in group K1 did not differ significantly from group K2 with a p-value of 0.075 (p0.05). Based on the data above, it can be concluded that the administration of hypoxic mesenchymal stem cell secretome at doses of 250 µl and 500 µl had a significant effect on MCP-1 expression in STZ-induced obese rats so the hypothesis statement was accepted.

IV. DISCUSSION

The sample size used was 20 male Wistar rats which were divided into 4 groups of 5 rats each, namely the control group (K1) with standard feed and distilled water, the second treatment group (K2) in STZ-induced obese rats, the third treatment (K3) was given hypoxic mesenchymal stem cell secretome at a dose of 250 µl in STZ-induced obese mice, and the fourth treatment group (K4) was given hypoxic mesenchymal stem cell secretome at a dose of 500 µl in STZ-induced obese mice. This study used male Wistar rats because they are similar to humans in terms of physiology, anatomy, and many human symptoms and conditions that can be replicated in mice.

The desired mice are mice with high blood glucose levels (hyperglycemia) except for group 1 (normal control). Mice with hyperglycemia were treated with a high-fat diet for 30 days and continued with STZ induction. Fat can cause diabetes mellitus because insulin stimulates lipogenesis in adipose tissue provides acetyl-CoA and NADPH needed for fatty acid synthesis and provides glycerol which is involved in triacylglycerol synthesis. Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, decreased insulin receptor sensitivity, or both.[1] DM sufferers experience higher oxidative stress[2], resulting in increased modification of lipids, DNA, and proteins in various tissues.

The results of the examination of TNF-α expression in the K2 group in STZ-induced obese rats without administration of hypoxic mesenchymal stem cell secretome experienced a significant increase compared to the control group (K1), the group that was given hypoxic mesenchymal stem cell secretome at a dose of 250 µl (K3) and 500 µl (K4) as in table 5.1. Streptozotocin was administered at a dose of 30 mg/kg BW intraperitoneally. Streptozotocin inhibits the Krebs cycle and reduces oxygen consumption in mitochondria, resulting in DNA damage that can activate poly ADP-ribosylation, which then results in suppressing cellular NAD+, further decreasing the amount of ATP, and finally inhibits insulin secretion and synthesis. Metabolic disorders with the occurrence of insulin receptor resistance and reduced ability of pancreatic β-cells to secrete insulin, there will be an increase in adipose tissue which causes hypertrophy and hyperplasia.[2] This situation triggers inflammation involving the role of pro-inflammatory cytokines and chemokines such as TNF-α (Tumor Necrosis Factor-Alpha).[3]

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TNF-α expression in obese rats induced by STZ and given hypoxic mesenchymal stem cell secretome at doses of 250 µl (K3) and 500 µl (K4) decreased as shown in Table 5.1. Administration of hypoxic mesenchymal stem cell secretome has been shown to inhibit damage caused by free radicals and can reduce TNF-α expression.[9] Hypoxic mesenchymal stem cell secretome is a potential and effective candidate in modulating the inflammatory environment and immune cells because it can produce anti-fibrotic, immunomodulating, and anti-apoptotic factors.[5]

The results of examining MCP-1 expression in the K2 group in STZ-induced obese rats without being given hypoxic mesenchymal stem cell secretome showed a significant effect compared to the group given hypoxic mesenchymal stem cell secretome at doses of 250 µl (K3) and 500 µl (K4) as in table 5.1. Type 2 diabetes mellitus is closely related to inflammatory conditions. One of the inflammatory markers in T2DM is chemokine monocyte chemoattractant protein-1 (MCP-1). Regulation of MCP-1 expression involves several mechanisms. Nuclear factor κB (NFκB) binding to the MCP-1 promoter region is an important mechanism for MCP-1 transcription. Epigenetic modification is another potential mechanism for regulating MCP-1 expression.

MCP-1 expression in STZ-induced obese mice and given hypoxic mesenchymal stem cell secretome at doses of 250 µl (K3) and 500 µl (K4) increased as shown in Table 1. Administration of hypoxic mesenchymal stem cell secretome has been shown to inhibit damage caused by free radicals and can reduce TNF-α expression.[9] The secretome of hypoxic mesenchymal stem cells does not differentiate in vivo into skeletal tissue but instead functions as a paracrine, i.e., a secretory center at the site of injury. These stem cells are capable of producing proteins and cytokines for tissue repair, immunomodulatory substances that can suppress inflammation due to injury or rejection of tissue transplants by the body as well as proangiogenic properties that have potential for therapy. Further research still needs to be done using a high-fat diet dose according to the body weight of the rats and It is necessary to carry out a fat analysis examination after treatment.

V. CONCLUSION

The administration of SSPM Hypoxia at a dose of 250 µl and 500 µl can decrease the TNFα and MCP-1 gene expression in obese male Wistar rats induced with STZ.

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