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The Effect of Secretome Hypoxia Mesenchymal Stem Cells (SH-MSCs) Cream on IL-6 Gene Expression (In Vivo Study on Psoriasis-like Rats Model Induced by SH-MSCs)

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ABSTRACT: Psoriasis is caused by chronic inflammation of the skin that occurs in various countries. Current therapy is still dominated by long-term anti-inflammatory which causes various side effects. Hypoxic secretome mesenchymal stem cells (SH-MSCs) contain soluble molecule bioactive anti-inflammatory cytokines that can suppress inflammation. However, the role of SH-MSCs on IL-6 gene expression in psoriasis-like has not been studied until now. The aim of this study was to determine the effect of SH-MSC administration on IL-6 gene expression in Psoriasis-like rats induced by IMQ 100 mg. The research design is an experimental posttest control group design. The experimental subject was divided into four treatment groups consisting of a healthy group (no treatment), positive control group (only IMQ 100 mg), P1 group (SH-MSC therapy 100 µL/kgBW in 100 mg cream) and P2 group (SH-MSCs therapy 200 µL/kgBW in 100 mg cream). P1 and P2 were given SH-MSC topically for 14 days. At the end of the treatment, termination, extraction skin and IL-6 gene expression analysis by qRT-PCR. The results of this study showed that the mean expression of the IL-6 gene in the healthy group was: 1.00±0.00; positive control: 6.16±1.19, P1: 3.06±1.65, P2: 1.06±0.41. Analysis data using Kruskal Wallis test with $p<0.05$ and Mann Whitney $p<0.05$ so that there was a significant difference between treatment group. The administration of SH-MSC had a significant effect on improving conditions in psoriasis-like model rats as indicated by a decrease in IL-6 gene expression.

KEYWORDS: IL-6 gene, SH-MSCs, psoriasis

I. INTRODUCTION

Psoriasis is a skin disease caused by chronic inflammation that occurs in countries around the world. Until now the treatment of Psoriasis is still dominated by anti-inflammatory long-term use which causes various side effects.[1–3]

The prevalence of Psoriasis in adults in the United States is 0.51% in a population of 799,607 patients with disabilities and/or over 65 years of age. The highest prevalence in the world was reported in Norway, namely as much as 11.43% in a population of 10,302 people from Tromso. Among 12 studies in Europe, the countries with the lowest and highest prevalence were reported in the UK 1.3% and Norway 11.43% respectively. Three studies in Australia showed a prevalence between 2.30% - 6.60%. The prevalence of psoriasis in adults in Brazil is around 1.30%. The prevalence of psoriasis in Indonesia reaches 2.5% of the population and there are still many who have not received medical treatment.[4,5]

In psoriasis, IL-6 is produced by keratinocytes, fibroblasts, endothelial cells, dendritic cells, macrophages, and type 17 T helper cells, and has been shown to exert many biologic effects in affected tissues, including (i) keratinocyte growth, activation, and production proinflammatory cytokines/chemokines (especially those synergizing with TNF-a and IL-17A). The proinflammatory cytokine IL-6, signaling via STAT-3, allows Tmem/eff cell release of Treg-mediated suppression in the murine system. IL-6 protein is highly increased and most highly expressed by CD31+ endothelial cells and CD11c+ dermal dendritic cells (DC) in psoriasis skin lesions. Elevated levels of IL-6 in the skin and serum are characteristic of psoriasis. Serum IL-6 level is considered as a marker of inflammatory activity in psoriasis as well as an indicator of treatment response.[4,5]

Mesenchymal Stem Cell (MSC) secrete soluble factors, referred to as Secretomes, which play a multifactorial role in the regulation of circulating inflammatory cells. For example, the secretome contains TGF-β and IL-10, which block T-cell proliferation. It is also

believed that these soluble factors are able to change the secretion profile of dendritic cells to increase the production of anti-inflammatory cytokines including IL-10 and decrease the production of inflammatory cytokines including IFN-γ.[6]
The role of the Secretome Hypoxia Mesenchymal Stem Cell (SH-MSC) in psoriasis has not been studied, so this study wanted to investigate the role of the secretome as seen from the expression of the IL-6 gene in Wistar rats with psoriasis by administering the topical cream with SH-MSC concentration of 100 µL/kgBW in 100 mg cream and 200 µL/kgBW in 100 mg cream.

II. MATERIAL AND METHOD

Experimental Subject

A 24 male Wistar rats with 8-12 weeks of age and 200-250 grams of body weight. The rats were clean-shaven on the dorsal and smeared Imiquimod cream dose of 100 mg for 10 days, so that a psoriasis-like condition was validated through macroscopic and microscopic examination. The rats were divided into a healthy group, a control group where psoriasis-like rats were given standard cream and the treatment group was given secretome cream.

MSCs Isolation and Cultivation

MSCs was isolated from the umbilical cord of 19-day-old pregnant rats carried out in the biosafety cabinet class 2 SCCR laboratory Faculty of Medicine Universitas Islam Sultan Agung Semarang. Samples were then collected in sterile culture washed with 0.9% NaCl and phosphate-buffered saline (PBS), The Wharton jelly that separated from the umbilical cord of blood vessels chopped into small fragments. The fractions were then evenly placed in the T25 flask until they adhered to the bottom of the flask for 2-3 minutes. After that, soaked with 1 cc of medium, incubated at 37°C, pH 7, 20% O_2 and 5% CO_2, then the medium flask was removed and replaced every three days in 10-15 days. Cell growth was observed using an inverted microscope and repeated until it reached 80% confluent stem cells. After that the cells were separated using BDTM. MSCs were incubated under hypoxic conditions with an O_2 concentration of only 2.5% for 2 hours indoors.

SH-MSCs Dose

Positive control group received IMQ 100 mg induced with standard cream, P1 received SH-MSC therapy 100 µL/kgBW in 100 mg cream and P2 received SH-MSC therapy 200 µL/kgBW in 100 mg cream. The healthy group did not receive any treatment. The MSCs were given for 14 days.

Rats Termination

At the end of the treatment, termination was carried out using a lethal dose cocktail of 10 ml containing Ketamine 50 mg/kgBW, Xylazine 10 mg/kgBW and Acepromazine 2 mg/kgBW which were injected intramuscularly. Once it's done extraction skin and IL-6 gene expression analysis by qRT-PCR.

Data Analysis

Normality test using the Shapiro Wilk test. The distribution of data in this study was not normal ($p < 0.05$) so the Kruskal Wallis test was performed. The results of the Kruskal Wallis test obtained a p value <0.05 then continued with the Mann Whitney test to find out the differences between group.

RESULT

Characteristics and Differentiation Mesenchymal Stem Cells (MSCs)

MSCs use an umbilical cord of 19 days-pregnant rats. the isolation and cultivation carried out after the 5th pasase which cell showed attached to the bottom of the flask with spindle-like cell morphology (Figure 1A). Flow cytometry Quantitative test results are obtained the percentage of positive expression of CD90.1 was 97.8%, CD29 was 99.0%, negative expression of CD45 was 2.0%, and CD31 was 2.1% (Figure 2). MSCs were tested for differentiation to determine their ability to become adipogenic and osteogenic derivatives (Figures 1B and 1C).

Figure 2. Detection of CD90.1, CD29 Surface Markers, Negative Expression of CD45, and CD31

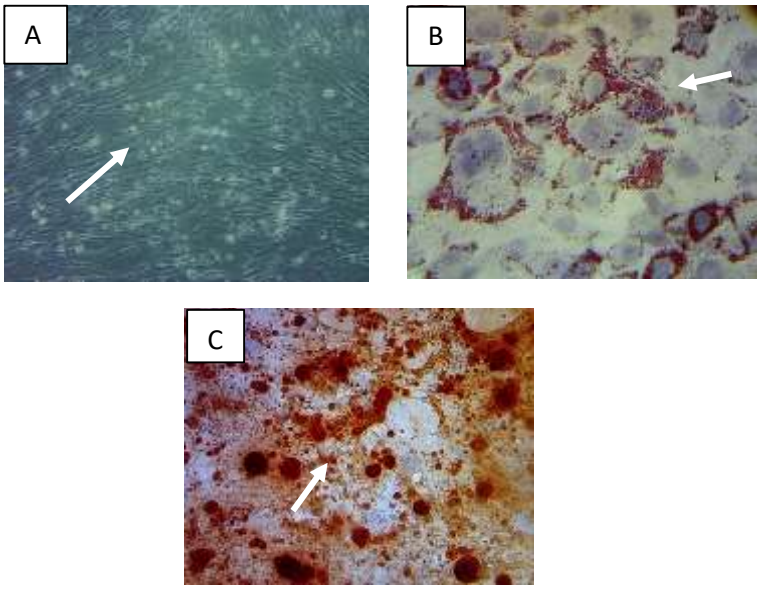

Figure 1. (A) MSCs validity test with a similar shape with fibroblasts at 200x magnification (B) MSCs validity test of adipogenic differentiation at 200x magnification (C) MSCs validity test of osteogenic differentiation at 200x magnification

Mesenchymal Stem Cells (MSCs) which was incubated under the hypoxic conditions with 5% concentration of O_2 for 24 hours using hypoxia chamber contained more cytokines interleukin-10 or IL-10 and Transforming growth factor-β, where there was more spindle-like (Figure 3).

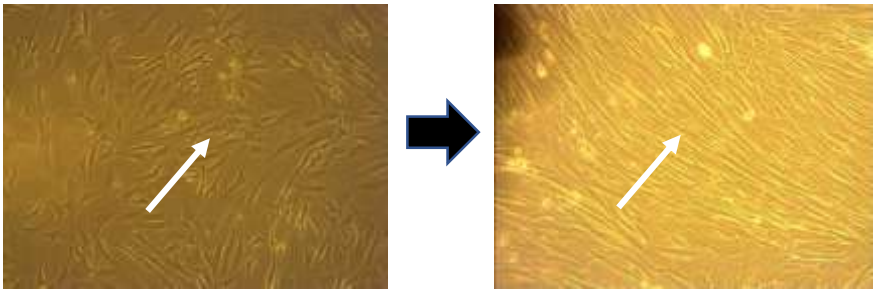

Figure 3. Different visualization of hypoxic mesenchymal stem cells

Validity test of Psoriasis-Like Rats

Validity test result of psoriasis-like rats showed that the administration of IMQ 100 mg can induce psoriasis-like which showed a significant increase in erythema, scale, and thickness, as well as a psoriasis-like appearance on the microscopic visualization (Figure 4).

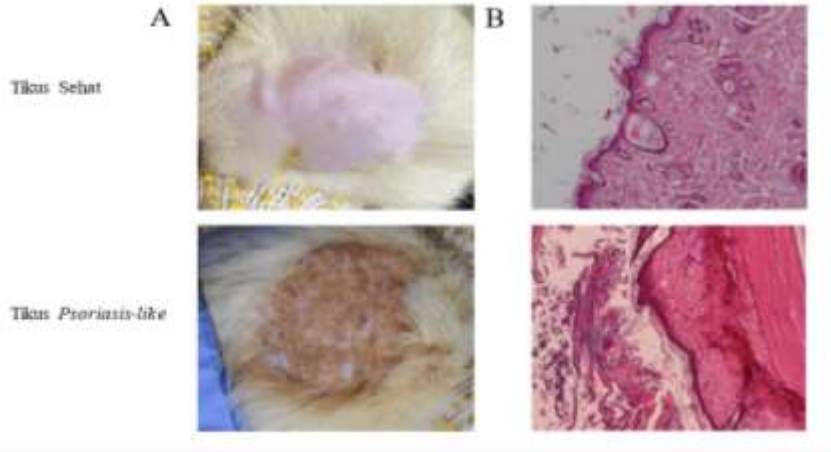

Figure 4. Validity test Psoriasis-like rats (A) Macroscopic appearance and (B) Microscopic images of healthy rats and rats induced with IMQ 100 mg showing signs of psoriasis-like

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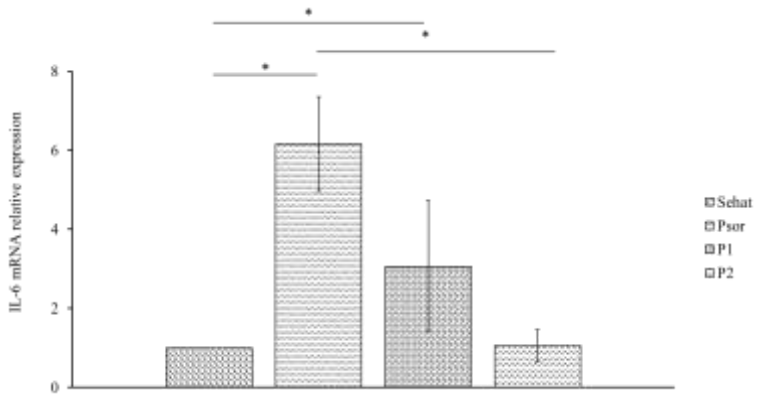
Expression of IL-6

The measurement of IL-6 expression in mRNA skin tissue of psoriasis-like rats using qRT-PCR method and calculated using the Livak method to determine the qRT value. In this study, there was a decrease in IL-6 gene expression in the group that received SH-MSC cream compared to the group that did not receive SH-MSCs cream. The decrease of IL-6 gene expression for group that given SH-MSC cream at a dose of 200 µL/kgBW in 100 mg cream was more significant than the SH-MSC group at a dose of 100 µL/kgBW in 100 mg cream (Table 1 and Figure 5).

Table 1. Data on IL-6 Gene Expression Research

Variable			Group		p-value
	Shamn=6	**KP**	**P1**	**P2**	
	Mean±SD	**n=6**	**n=6**	**n=6**	
		Mean±SD	**Mean±SD**	**Mean±SD**	
Expression contains IL-6	1.00±0.00	6.16±1.19	3.06±1.65	1.06±0.41	
Sapphire wilk	0.00	0.872	0.972	0.910	
Kruskal Wallis				0.000	



Picture 5. RT-PCR results of IL-6 gene expression level ratio.

*** Shows a significant difference between the healthy, positive, treatment 1 and 2 groups (p<0.05).**

III. DISCUSSION

Mesenchymal Stem Cells (MSCs) secrete a soluble factor, known as Secretom, which plays a multifactorial role in the regulation of circulating inflammatory cells which contain TGF-β and IL-10. There are studies that reveal MSC administration can increase anti-inflammatory factors and immuno-suppressant such as interleukin-10 (IL-10), hepatocyte growth factor (HGF), transforming growth factor-β (TGFβ1), and prostaglandins. E2 (PGE2) which can act as an anti-inflammatory.[7-12] This study aims to determine the effect of topical application of various doses of SH-MSC cream on the expression of the IL-6 gene which plays a role in Psoriasis Like. This study used male rats of the Wistar strain because they included vertebrate mammals with structures of organs and tissues similar to humans. Test animals were induced by IMQ 5% dose of 100 mg to make Psoriasis Like conditions.[13]

Expression IL-6 functions as a mediator for the notification of the occurrence of several emerging events such as autoimmunity and tissue damage. In addition to immune-mediated cells, mesenchymal cells, endothelial cells, fibroblasts, and many other cells are involved in IL-6 production in response to various stimuli.[14] Increased levels of IL-6 in the skin and serum are markers of an inflammatory process that occurs in cases of psoriasis. In the case of psoriasis, IL-6 is produced by various cell types in psoriatic plaques, including keratinocytes, fibroblasts, endothelial cells, DCs, and macrophages. The produced IL-6 further augments the IL-6-rich microenvironment in psoriasis plaques that can induce itpSTAT3 in Th17 effector and memory cells. signalingpSTAT3 persistence in T cells is required for early Th17 differentiation and promotion of Th17 cytokine production, unleashing uncontrolled activation of effector T cells, and preventing the suppressive activity of regulatory T cells.(14) This process can also promote keratinocyte growth and proliferation, and promote hyperplasia epidermal psoriasis.[15]

In this study, SH-MSC cream was given by topical significantly and dose-dependently decreased IL-6 gene expression in psoriasis-like mouse skin. In giving SH-MSC cream dose of 100 µL/kgBW in 100 mg cream can decrease expression the IL-6 gene was up to 3.06 ± 1.65 while at the SH-MSC cream dose of 200 µL/kgBW in 100 mg cream the IL-6 gene expression decreased by 1.05 ± 0.41

with the control group as high as 6.16 ± 1.19. Based on the results of this study, showing that administration of SH-MSC cream can reduce inflammation in psoriasis-like cases induced by IMQ through down regulation expression of the proinflammatory cytokine IL-6.

This research needs to be validation autoimmune markers test. The variations of intervention in the form of standard drug administration psoriasis-like compared with therapy using SH-MSC, and variations in the time of administration after being induced Psoriasis-like are needed in order to determine the potential for prevention or cure psoriasis-like which is more optimal.

IV. CONCLUSION

The administration of SH-MSC cream at a dose of 100 µL/kgBW in 100 mg cream and a dose of 200 µL/kgBW in 100 mg cream had an effect on decreasing IL-6 gene expression in male rats of the Wistar strain model psoriasis-like between treatment groups compared to controls.

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