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Effect of SH-MSC on IL-10 and STAT3 Gene Expression in Infected Wounds

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Abstract

Superficial wounds infected with *Staphylococcus aureus* trigger inflammatory responses and oxidative stress that impair wound healing. The secretome from hypoxia-conditioned mesenchymal stem cells (SH-MSC) has been shown to modulate inflammation by upregulating anti-inflammatory mediators, including IL-10 and STAT3. This study aimed to evaluate the effect of SH-MSC gel on IL-10 and STAT3 gene expression in superficial wounds infected with *Staphylococcus aureus*. This experimental study was conducted at the Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, Indonesia from January to February 2024. A post-test control group design was used, with Wistar rats divided into five groups. Groups K2, K3, P1, and P2 consisted of Wistar rats with superficial wounds infected with *Staphylococcus aureus*, while K1 served as the healthy control. K2 was the negative control (treated with 100 mg of base gel), and K3 was the positive control (treated with topical gentamicin). In the experimental groups, P1 received 25% SH-MSC gel, while P2 was treated with 50% SH-MSC gel. The expression of IL-10 and STAT3 genes was analyzed using the Mann–Whitney test. The results showed a significant increase in IL-10 gene expression in P1 ($p=0.048$) and P2 ($p<0.001$) when compared with K2. The STAT3 gene expression was also significantly higher in P1 and P2 than in K3 ($p<0.001$). These findings indicate that SH-MSC gel upregulates IL-10 and STAT3 gene expression in Staphylococcus aureus-infected superficial wounds, suggesting an anti-inflammatory mechanism of action.

Keywords: Hypoxia, infected wound, interleukin-10, mesenchymal stem cells, STAT3

Introduction

Superficial skin wounds are highly susceptible to bacterial infection when the integrity of the skin barrier is disrupted, making wound infection a common clinical challenge. Staphylococcus aureus is one of the predominant pathogens in these infections and is known to prolong inflammation and delay tissue repair.[1] During bacterial infection, excessive production of reactive oxygen species (ROS) contributes to oxidative stress and sustains inflammatory signaling, ultimately impairing normal wound healing processes.[2]

Mesenchymal stem cell (MSC) secretome is a cell-free therapeutic option that exerts anti-inflammatory and immunomodulatory effects through paracrine signaling. Hypoxic preconditioning increases the expression of bioactive factors in MSC secretome, yielding hypoxia-conditioned MSC secretome (SH-MSC) with greater regenerative potential. Previous studies have shown that MSC-derived conditioned media accelerate wound closure and enhance tissue regeneration in various wound models.[3,4]

Despite these promising findings, the molecular mechanisms underlying the effects of SH-MSC on infected wound healing, particularly in Staphylococcus aureus-associated wounds, remain insufficiently understood. Specifically, no study has examined how SH-MSC modulates Interleukin-10 (IL-10) and Signal Transducer and Activator of Transcription 3 (STAT3) signaling in this infection model. IL-10 suppresses excessive inflammation, while STAT3 regulates cell proliferation, angiogenesis, and macrophage polarization during tissue repair.[5,6]

Therefore, this study evaluated the effect of topical SH-MSC gel (25% and 50%) on IL-10 and STAT3 gene expression in Wistar rats with Staphylococcus aureus-infected superficial wounds.

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Methods

This experimental study employed a post-test only control group design and was conducted at the Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, Indonesia, from January to February 2024. All animal procedures were approved by the Ethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia (Ethical Clearance No. 9/I/2024/Komisi Bioetik) and were performed in accordance with institutional guidelines for the care and use of laboratory animals. A total of 30 male Wistar rats were included in the study and randomly assigned into five groups (n=6 per group): healthy control, negative control, positive control, 25% SH-MSC gel treatment, and 50% SH-MSC gel treatment (Figure 1). Mesenchymal stem cells (MSCs) were isolated from the umbilical cords of 19-day gestational rats. Cell characterization was performed by evaluating osteogenic and adipogenic differentiation potential and by assessing cell-surface marker expression using flow cytometry. Following validation, the MSCs were cultured under hypoxic conditions to obtain hypoxia-conditioned MSC secretome for subsequent experimental procedures.

Thirty healthy male Wistar rats aged 2–3 months and weighing 250±10 g were obtained from a licensed breeder in Semarang. The animals were acclimatized for seven days under standard laboratory conditions with a 12-hour light–dark cycle and free access to food and water. Only healthy male rats without visible skin or morphological abnormalities were included, while rats that failed to develop infected wounds after bacterial inoculation or had been previously used in other experimental procedures were excluded.

Staphylococcus aureus was obtained from the SCCR Laboratory collection. The bacterial strain

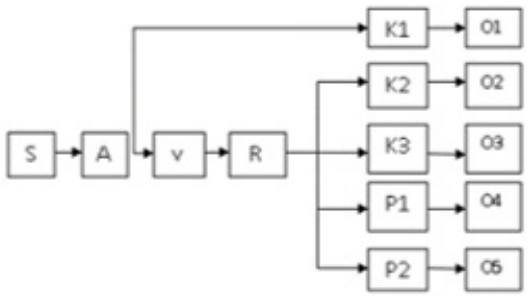

Figure 1 Post-test Only Control Group Design

was cultured on nutrient agar and incubated at 37°C for 24 hours. Bacterial colonies were suspended in sterile phosphate-buffered saline (PBS) and adjusted to a concentration of 5×10^7 CFU/mL. The dorsal fur of the Wistar rats was shaved, and a 2×2 cm area was marked on their backs. A 1-mm deep incision was made using a scalpel, and immediately after, a 100 µL suspension of Staphylococcus aureus at a concentration of 5×10^7 CFU was applied to induce infection. Inclusion criteria required rats to be healthy and free from morphological abnormalities, with infected superficial wounds validated through Gram staining. Rats that did not develop infected wounds or had been used in previous studies were excluded.

Sample size was determined according to WHO recommendations for experimental animal studies, requiring a minimum of five animals per group. The rats were randomly assigned to five groups (Figure 2): Group K1 (healthy control), Group K2 (infected wounds treated with 100 mg base gel), Group K3 (infected wounds treated with topical gentamicin), Group P1 (treated with 25% SH-MSC gel), and Group P2 (treated with 50% SH-MSC gel). SH-MSC were obtained from rat umbilical cord-derived mesenchymal stem cells, cultured in a hypoxic chamber using DMEM supplemented with FBS, PBS, fungizone, and penstrep. The culture media were filtered using Tangential Flow Filtration (TFF) to obtain SH-MSC, which were further validated using ELISA to confirm VEGF, PDGF, FGF, TGF-β, and IL-10 levels. The application of SH-MSC gel was performed using sterile swabs.

Skin tissue samples were collected on day 4 after treatment. Tissue specimens were processed for histological evaluation using hematoxylin-eosin and Gram staining. Total RNA was extracted from wound tissue samples, followed by complementary DNA synthesis. The expression levels of IL-10 and STAT3 genes were quantified using quantitative real-time polymerase chain reaction (qRT-PCR). Relative gene expression was calculated after normalization against an appropriate housekeeping gene.

Data were analyzed using SPSS software. Normality was assessed using the Shapiro–Wilk test, and variance homogeneity was evaluated using Levene's test. Because the data were not normally distributed, comparisons between groups were performed using non-parametric statistical tests, including the Mann–Whitney test. Statistical significance was defined as $p<0.05$.

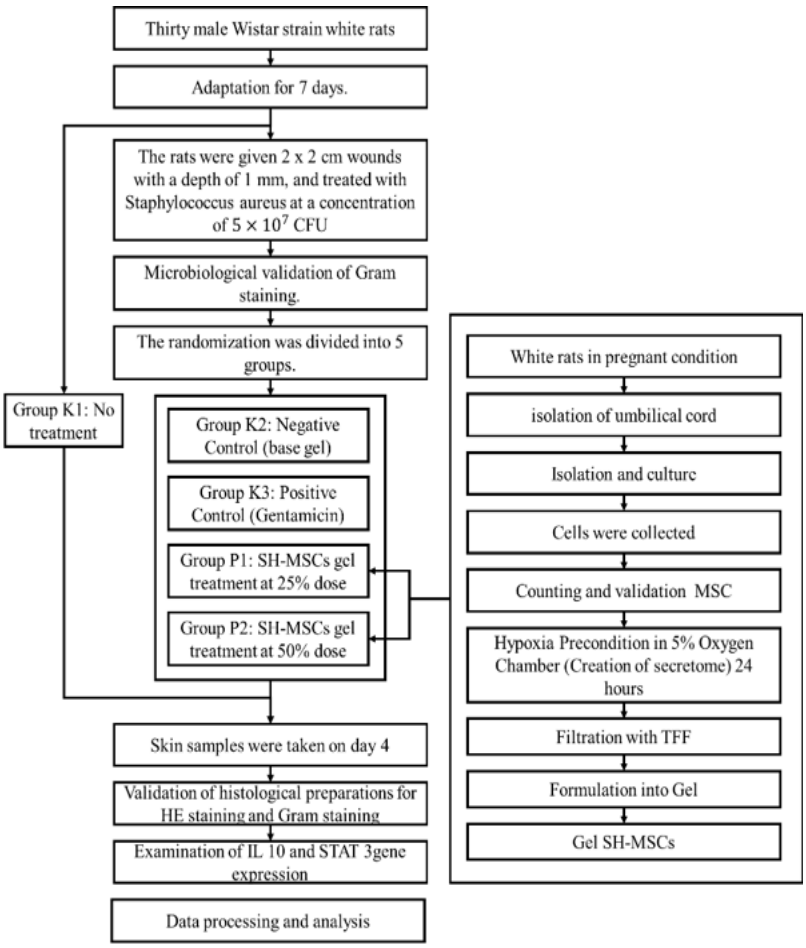

Figure 2 Research Design

Results

The mean expression levels of IL-10 and STAT3 genes in each experimental group are presented in Table 1. The highest IL-10 expression was observed in the P2 group (1.99±0.44), whereas the lowest expression was found in K2 (0.45± 0.21). Similarly, STAT3 expression was highest in P2 (2.41±1.12) and lowest in K3 (0.21±0.12).

Normality testing using the Shapiro–Wilk test demonstrated that several datasets were not normally distributed (Table 2). Therefore, non-parametric statistical analyses were applied. Kruskal–Wallis testing revealed significant differences in both IL-10 and STAT3 gene expression among the experimental groups (both $p<0.001$).

Pairwise comparisons using the Mann–Whitney test are presented in Table 3. For IL-10 expression, significant differences were observed between K1 and K2 ($p=0.004$), K2 and P1 ($p=0.048$), K2 and P2 ($p<0.001$), K3

Table 1 Mean±Standard Deviation of IL-10 Gene Expression and STAT3 Gene Expression

Variable	IL-10 Gene Expression	STAT3 Gene Expression
K1	1.00±0.00	1.00±0.000
K2	0.45±0.21	0.45±0.14
K3	0.73±0.19	0.21±0.12
P1	0.90±0.32	1.78±0.72
P2	1.99±0.44	2.41±1.12

Table 2 Shapiro–Wilk Test Results for IL-10 and STAT3 Gene Expression

Variable	IL-10 Gene Expression	STAT3 Gene Expression
K1	p<0.001	p<0.001
K2	p=0.549	p=0.580
K3	p=0.034	p=0.635
P1	p=0.718	p=0.960
P2	p=0.747	p=0.392

and P2 (p=0.001), and P1 and P2 (p=0.013). For STAT3 expression, significant differences were observed between K1 and K3 (p=0.007), K2 and P1 (p=0.010), K2 and P2 (p=0.002), K3 and P1 (p<0.001), and K3 and P2 (p<0.001). The distribution of IL-10 and STAT3 gene expression across the experimental groups is presented in Figure 3. The highest expression levels for both biomarkers were observed in the P2 group..

The Kruskal–Wallis test confirmed a significant difference in STAT3 expression among groups (p<0.001). Topical application of SH-MSC gel at both 25% and 50% concentrations significantly upregulated IL-10 and STAT3 gene expression in Staphylococcus aureus-infected superficial wounds, with the 50% concentration producing the highest expression levels.

Discussion

Both IL-10 and STAT3 gene expression increased following topical SH-MSC gel treatment, with the highest levels observed in the P2 group (50% concentration), suggesting a concentration-dependent response. The increased expression of IL-10 in both treatment groups indicates a potential anti-inflammatory effect of SH-MSC

Table 3 Results of Mann Whitney IL-10 and STAT3 Gene Expression

Variable	Group A	Group B	p-value	Significant (*)
IL-10	K1	K2	0.004	*
	K1	K3	0.100	
	K1	P1	0.348	
	K1	P2	0.122	
	K2	K3	0.205	
	K2	P1	0.048	*
	K2	P2	0.001	*
	K3	P1	0.479	
	K3	P2	0.001	*
	P1	P2	0.013	*
STAT3	K1	K2	0.096	
	K1	K3	0.007	*
	K1	P1	0.357	
	K1	P2	0.147	
	K2	K3	0.307	
	K2	P1	0.010	*
	K2	P2	0.002	*
	K3	P1	0.001	*
	K3	P2	0.001	*
	P1	P2	0.059	

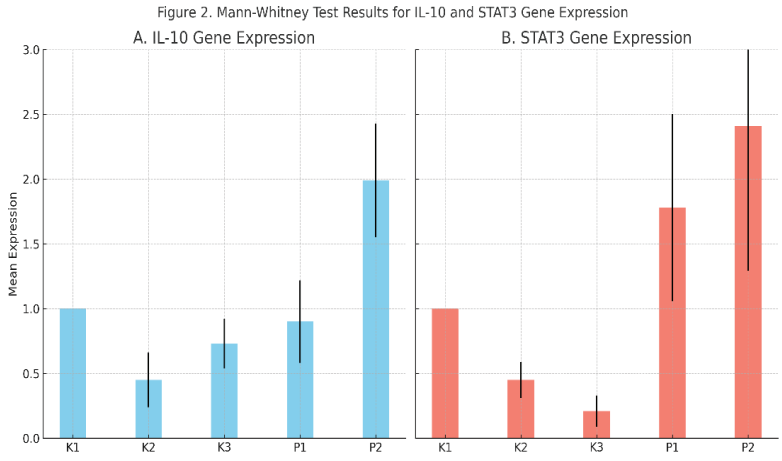

Figure 3 Distribution of IL-10 and STAT3 Gene Expression Across Experimental Groups

gel. IL-10 is a key anti-inflammatory cytokine that suppresses excessive inflammatory responses and contributes to the resolution phase of wound healing. The greater expression observed in the P2 group suggests that higher concentrations of SH-MSC gel may provide enhanced immunomodulatory effects.STAT3 expression was also increased elevated in the treatment groups compared with the infected control groups. STAT3 plays an important role in tissue repair, angiogenesis, cell proliferation, and regulation of inflammatory responses during wound healing. The concurrent increase in IL-10 and STAT3 expression following SH-MSC treatment suggests the possible involvement of the IL-10/STAT3 signalling pathway in the healing response. However, because STAT3 activation and downstream signalling molecules were not directly evaluated, the involvement of this pathway should be interpreted cautiously.

These present findings are generally consistent with previous studies investigating MSC-derived secretomes in tissue regeneration. Suhandi et al. demonstrated the beneficial effect of MSC secretome in wound healing using a systematic review and meta-analysis design without a bacterial infection model, whereas the present study used an in vivo infected wound model with gene expression as the primary outcome. Yang et al. reported hat hypoxia-conditioned MSCs produced increased levels of growth factors and anti-inflammatory mediators that enhanced tissue regeneration. Putra et al. demonstrated accelerated wound closure after topical MSC-conditioned medium, but employed a full-thickness, non-infected wound model, which differs from the present study in wound depth, infection status, and outcome measures, limiting direct comparison. Collectively, the data support the view that SH-MSC gel may improve infected wound healing through the IL-10/STAT3 pathway.

Several limitations should be considered when interpreting these findings. First, the anti-inflammatory effects of SH-MSC gel were evaluated using IL-10 and STAT3 gene expression only, while other important inflammatory mediators such as TNF-α and IL-6 were not assessed. Second, protein expression levels were not measured; therefore, the relationship between gene expression and functional protein activity could not be confirmed. Third, histopathological evaluation and quantitative wound-healing parameters were not included, limiting assessment of the biological significance of the observed molecular changes. Future studies should incorporate additional inflammatory biomarkers, protein-level analyses, histopathological assessment, and wound-healing outcomes to better clarify the mechanisms underlying the therapeutic effects of SH-MSC gel..

In conclusion, topical SH-MSC gel increased IL-10 and STAT3 gene expression in Staphylococcus aureus-infected superficial wounds, with the highest expression observed at the 50% concentration. These findings suggest that SH-MSC gel may contribute to modulation of inflammatory responses and tissue-repair processes during infected wound healing. Further

studies are required to confirm the underlying mechanisms and evaluate its potential as a cell-free regenerative therapy.

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