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ORIGINAL ARTICLE

Hypoxia-preconditioned mesenchymal stem cells attenuate proinflammatory cytokines in collagen loss animal model

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ABSTRACT

BACKGROUND

Repeated ultraviolet-B (UVB) exposure induces significant collagen degradation, primarily through overproduction of reactive oxygen species, which subsequently drives an inflammatory cascade. Hypoxia-preconditioned mesenchymal stem cells (H-MSCs) constitute a promising therapeutic approach to counteract collagen loss by modulating inflammatory pathways. This study aimed to evaluate the potential of H-MSCs in regulating NF-κB p65 and IL-1β expression in a collagen loss rat model, highlighting their therapeutic efficacy.

METHODS

Twenty-five healthy male Wistar rats were randomly assigned to five groups: K1 (healthy controls), K2 (collagen loss), K3 (collagen loss + hyaluronic acid), K4 (collagen loss + 2.5×10^5 H-MSCs), and K5 (collagen loss + 5×10^5 H-MSCs). Collagen loss was induced by UVB radiation (peak wavelength: 302 nm) for 2 weeks. mRNA expression of NF-κB p65 was quantified by qRT-PCR, while IL-1β levels were assessed using ELISA. The rats were maintained for 14 days before being sacrificed, to allow the H-MSCs to exert their therapeutic effects. Data analysis was by One-way ANOVA with Tukey's post-hoc test.

RESULTS

The administration of H-MSCs significantly reduced IL-1β levels in groups K4 (633.14±63.76 pg/mL) and K5 (520.80±123.82 pg/mL) compared to group K2 (931.93±205.80 pg/mL) ($p<0.05$), with group K5 showing the most substantial reduction. Moreover, H-MSC injection in groups K4 and K5 effectively reduced NF-κB p65 expression levels (1.13±0.50 a.u. and 0.72±0.22 a.u., respectively), compared to group K2 (2.47±0.50 a.u.) ($p<0.05$), with group K5 providing optimum inhibition.

CONCLUSION
This study demonstrated that H-MSCs effectively attenuate UVB-induced inflammation and modulate key inflammatory pathways.

Keywords: Collagen loss, H-MSCs, p65, IL-1β, rats

INTRODUCTION

Repeated exposure to ultraviolet-B (UVB) radiation leads to significant collagen loss, characterized by reduction in epidermal thickness, degradation of elastic fibers, and increased skin wrinkling and dryness. This damage compromises the extracellular matrix (ECM), primarily composed of structural proteins such as collagen and elastin, which are crucial for maintaining skin elasticity.[1] UVB-induced collagen degradation is closely associated with the overproduction of reactive oxygen species (ROS), which trigger proinflammatory pathways, including the activation of nuclear factor kappa B (NF-κB) subunit p65 and elevation of interleukin-1β (IL-1β) levels.[3] This inflammatory cascade upregulates degradative enzymes that accelerate collagen degradation while suppressing collagen synthesis.[4] Current therapies, such as retinol and hyaluronic acid (HA), and clinical procedures such as laser resurfacing and botulinum toxin injections, present significant side effects and limitations.[5-7] Emerging evidence suggests that hypoxia-preconditioned mesenchymal stem cells (H-MSCs) may offer a novel therapeutic strategy for UVB-induced collagen loss.[8-12]

Hypoxia-preconditioned mesenchymal stem cells (H-MSCs) offer promising therapeutic potential in mitigating collagen loss by modulating inflammatory pathways.[13] Sourced from tissues such as the umbilical cord, bone marrow, and adipose tissue, H-MSCs are characterized by the surface markers CD105, CD90, and CD73, while lacking hematopoietic markers such as CD34 and CD45.[14,15] When cultured under hypoxic conditions, H-MSCs secrete a bioactive secretome enriched with growth factors and anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor beta (TGF-β), which enhance their regenerative and immunomodulatory capabilities.[16] These capabilities may contribute to attenuate collagen loss, by shifting the inflammatory response toward a reparative phase.

The proinflammatory mediators NF-κB subunit p65 and IL-1β are pivotal in triggering the pathological cascade that impairs ECM integrity and disrupts collagen synthesis.[4] Recent studies have highlighted that H-MSCs, through their capacity to secrete anti-inflammatory molecules, may mitigate the harmful effects of collagen loss, through modulating proinflammatory conditions, thereby restoring ECM homeostasis.[1] Notably, H-MSCs regulate the expression of α-smooth muscle actin (α-SMA) in myofibroblasts, a marker of tissue repair, by dynamically modulating wound healing phases.[4] While increased α-SMA expression facilitates wound closure, prolonged expression may result in abnormal collagen synthesis and scar formation, highlighting the need for precise regulation.[17] Similarly, H-MSCs play a critical role in suppressing proinflammatory conditions, ensuring that the healing process is optimized without progressing toward pathological conditions.[16] The study by Mayasari et al. [18]found that treatment with the secretome from hypoxic mesenchymal stem cells (H-MSCs) resulted in decreased expression of genes IL-6 (8.59 ± 3.32 pg/mL), p50 (4.35 ± 2.27), and p65 (4.09 ± 1.82 a.u.) in a hyperpigmentation animal model, compared to the control group. The decrease in gene expression was observed to correlate with increasing concentration of the H-MSC secretome.[18] While the previous study has shown the effects of the H-MSC secretome on IL-6, p50, and p65 gene expression in a hyperpigmentation model, studies investigating its role in collagen loss models have not yet been published.

The present study evaluated the effect of H-MSC-mediated suppression of inflammatory mediators in a UVB-induced collagen degradation model, with specific focus on quantitatively assessing the dose-dependent regulatory effects of H-MSCs on NF-κB p65 and IL-1β expression in vivo.

METHODS

Research design

This post-test-only experimental study was carried out at the Stem Cell and Cancer Research (SCCR) Laboratory, located in Semarang, Central Java, Indonesia, between October and November 2024.

Experimental animals

The study utilized Wistar rats (*Rattus norvegicus*) weighing 200–250 g as an animal model for collagen loss. They were acquired from the Animal Research Centre laboratory, SCCR Indonesia. Environmental conditions were carefully regulated. The indoor temperature was maintained at 26°C, with 12-hour light/dark cycles, and humidity of 50-60%. The animals had free access to food and water. The feed and bedding were regularly replaced to ensure healthy and stable growth. In this study, the sample size was determined based on Federer's formula: $(t - 1)(n - 1) \geq 15$, where t is the number of treatment groups and n is the number of replications per group. With five groups ($t=5$) and five rats per group ($n=5$), this study needed 25 male Wistar rats, each weighing between 200 and 250 g. The rats were randomly divided into five groups based on previous research.[8]

Preparation, phenotyping, and differentiation analysis of UC-MSCs

Umbilical cord-derived mesenchymal stem cells (UC-MSCs) were isolated following established protocols.[9] The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 1.5% penicillin/streptomycin (Gibco), and 0.25% amphotericin B (Gibco) at 37°C under 5% CO_2 conditions. The culture medium was replaced every three days. UC-MSCs at passage 5 were used for all subsequent experiments. Flow cytometry analysis was conducted to characterize surface markers on UC-MSCs at passage 5. Cells were stained with rat anti-CD90-FITC, CD29-PE, CD31-perCP, and CD45-APC antibodies (BD Bioscience, CA, USA). Analysis was performed using a BD Accuri C6 Plus flow cytometer and accompanying software (BD Bioscience). Adipogenic and osteogenic differentiation potential of UC-MSCs at passage 5 was assessed using standard differentiation media at 37°C with 5% CO_2. Adipogenic and osteogenic differentiation basal media (MesenCult™; Stem Cell Technologies, Singapore) were supplemented with specific differentiation supplements (Stem Cell Technologies), 1% L-glutamine (Gibco), 1% penicillin (Gibco), and 0.25% amphotericin B (Gibco). Media changes were performed every three days. After 21 days of differentiation, lipid deposits were visualized using Oil Red O staining, while calcium deposits were identified using Alizarin Red staining (Sigma-Aldrich, MO, USA).

Induction of hypoxic environment for UC-MSCs

To simulate hypoxic conditions, UC-MSCs at approximately 80% confluence were transferred to a hypoxia chamber (Stem Cell Technologies). The chamber's oxygen concentration was maintained at 5% using an oxygen controller (BioSpherix, Lacona, NY, USA), which continuously monitored the partial oxygen pressure (pO_2). The cells were incubated under these conditions for 18 hours at 37°C with 5% CO_2. After the incubation period, the culture medium was collected for subsequent analyses.

Generation of collagen loss animal model

A total of 25 healthy male Wistar rats, each weighing between 200 and 250 g, were provided with unrestricted access to food and water. The rats were maintained under controlled conditions at a temperature of 26 °C with a 12-hour light/dark cycle. After one week of acclimatization, the rats were randomly assigned to five groups: K1 (healthy rats), K2 (collagen loss), K3 (collagen loss + hyaluronic acid (HA)), K4 (collagen loss + 2.5×10^5 H-MSCs), and K5 (collagen loss + 5×10^5 H-MSCs), with five rats per group. UVB radiation with a peak emission wavelength of 302 nm (CL100M, UVP, USA) was employed to induce collagen loss. Following a previously established protocol, the rats were exposed to 160 mJ/cm² of UVB light for 30 minutes daily over five consecutive days. [19]

H-MSC administration

All animal procedures were conducted at the Animal Research Centre, SCCR Indonesia, in accordance with established ethical guidelines. H-MSCs were administered subcutaneously into rat skin in groups K4 and K5 by a veterinarian, using a single injection of 200 µL NaCl. Rats in group K4 received a dose based on previous research[20] of 2.5×10^5 H-MSCs, while those in group K5 were treated with 5×10^5 H-MSCs. Additionally,

rats in group K3 were administered 200 µL of HA, while groups K1 and K2 were treated with 200 µL NaCl. The injections were administered under sterile conditions to ensure precision and safety. Following administration, the rats were maintained for 14 days before being sacrificed for further analysis, to allow the H-MSCs to exert their therapeutic effects.

Sample collection

On day 15, the rats were anesthetized via intramuscular injection using a combination of ketamine (60 mg/kgBW) and xylazine (20 mg/kgBW) to ensure proper sedation. Following anesthesia, dorsal skin tissue samples were carefully harvested. The collected tissue samples were preserved in RNAlater® solution (Sigma-Aldrich) for subsequent qRT-PCR analysis to ensure RNA integrity. Additional samples were fixed in 10% buffered formalin for histological evaluation, including collagen staining. Remaining tissue samples were snap-frozen and stored at -80°C for intracellular enzyme-linked immunosorbent assay (ELISA) to quantify protein expression levels.

Collagen staining

Paraffin-embedded skin tissue blocks were sectioned into 5 µm thick slices using a precision microtome. The sections were stained with Masson's Trichrome stain (Bio-Optica, Milano, Italy) following the manufacturer's protocol to visualize collagen fibers. Stained slides were examined under a light microscope (Leica, Germany) at appropriate magnifications. The collagen density was quantified using ImageJ software, calculated as the percentage of collagenous tissue area relative to the total tissue area on each slide. This quantitative analysis ensured an accurate assessment of collagen deposition.

mRNA expression analysis

Total RNA was extracted from 50 mg of dorsal skin tissue collected on day 14 using TRI Reagent (Sigma-Aldrich), following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using the Enhanced Avian First Strand cDNA Synthesis Kit (Sigma-Aldrich), adhering to the manufacturer's instructions. The reverse transcription reaction utilized oligo (dT) primers, with an incubation step at 70°C for 10 minutes, followed by 45°C for 15 minutes. Quantitative real-time PCR (qRT-PCR) was performed in a two-step protocol using the Eco Real-Time PCR System (Illumina Inc., San Diego, CA, USA) and the KAPA SYBR® FAST Universal Kit (Sigma-Aldrich). A cDNA template of 3 ng was used for each reaction. The expression levels of p65 were assessed, with β-actin serving as the reference gene. The primer sequences were as follows: β-actin: forward 5'-GCCTTCCTTCCTGGGTATG-3' and reverse 5'-AGGAGCCAGGGCAGTAATC-3'; p65: forward 5'- AACACTGCCGAGCTCAAGAT-3' and reverse 5'-CATCGGCTTGAGAAAAGGAG-3'. The thermocycling conditions included an initial denaturation step at 95°C for 3 minutes, followed by 40 cycles at 95°C for 10 seconds and at 60°C for 30 seconds. Gene expression levels were quantified using the ΔΔCt method, analyzed through the EcoStudy Software (Illumina), and normalized to the β-actin housekeeping gene.

Intracellular ELISA

The intracellular levels of IL-1β were measured using a specific ELISA kit, catalog number … (Elabscience, Texas, USA) according to the manufacturer's protocol. Tissue lysates were prepared from the extracted samples using a radioimmunoprecipitation (RIPA) lysis buffer containing PMSF Protease Inhibitor (Cat No. E EL SR002), 0.25% Trypsin Solution, sodium fluoride, and a protease inhibitor cocktail. The lysates were centrifuged at 4°C for 10 minutes, and the supernatants were collected for analysis. Protein concentrations were determined using UV-Vis spectrophotometry at 260 nm and 280 nm to ensure sample quality and consistency. Equal volumes of protein from each sample were loaded into ELISA wells. Standard curves were generated for each assay to calculate IL-1β concentrations. Colorimetric absorbance was recorded at a wavelength of 450 nm using a microplate reader. All measurements were conducted in triplicate to ensure the reliability and reproducibility of the results.

Ethical clearance

All procedures involving animals adhered to the ethical standards and guidelines established by the Institutional Research Bioethics of Universitas Islam Sultan Agung, under approval number 421/X/2024/Komisi Bioetik.

Statistical analysis

All statistical analyses were conducted using SPSS version 26 (IBM, New York, USA). For data

following a normal distribution, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test to assess pairwise group differences. For non-normally distributed data, the Kruskal-Wallis test was employed, followed by multiple comparison test Mann-Whitney U tests for pairwise comparisons. Results were expressed as mean ± standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

RESULTS

H-MSC characteristics

The characteristics of H-MSCs at the fifth passage were assessed based on cell morphology, membrane marker expression, and differentiation potential, following the International Society for Cellular Therapy (ISCT) guidelines. Morphologically, the cells exhibited a fibroblast-like, spindle-shaped appearance (Figure 1a). Adipogenic (Figure 1b) and osteogenic (Figure 1c) differentiation assays confirmed their multilineage differentiation capacity. After 21 days of incubation, the cells successfully differentiated into adipocytes and osteocytes, as evidenced by lipid droplet accumulation and calcium deposition, respectively, visualized with red staining. Immunophenotyping revealed that the H-MSCs were positive for CD90 and CD29, characteristic markers of mesenchymal stem cells, while hematopoietic and endothelial markers CD45 and CD31 were not expressed, consistent with the H-MSCs' mesenchymal origin (Figure 1d).

UV-B exposure induces collagen loss in rats

In this study, we successfully established a UVB-induced collagen degradation model, as validated through both macroscopic and histological assessments. Macroscopic observations revealed visible skin discoloration, with brown pigmentation indicative of UVB-induced damage. Histological analysis using Masson's Trichrome staining further confirmed collagen loss in the dermal tissue, as evidenced by the reduced blue color in UVB-exposed samples (Figure 1). These findings demonstrate that UVB irradiation effectively impaired collagen synthesis, leading to significant collagen depletion. This validated animal model provides a robust platform for studying UVB-induced skin damage and potential therapeutic interventions.

H-MSC injection suppresses IL-1β levels in UVB-induced collagen loss in rat skin

Intracellular ELISA was performed to evaluate IL-1β levels in the skin tissue of the experimental groups. A significant elevation of IL-1β was observed in group K2 (931.94±205.80 pg/mL) compared to group K1 (203.99±51.76 pg/mL) ($p<0.05$), indicating increased inflammatory activity resulting from UVB exposure. The administration of H-MSCs in groups K4 (633.14±63.76 pg/mL) and K5 (520.80±137.81 pg/mL) significantly reduced IL-1β levels compared to group K2 (931.94±205.80 pg/mL) ($p<0.05$), demonstrating the anti-inflammatory effects of H-MSC treatment. Notably, group K5 (520.80±137.81 pg/mL), which received the higher dose of H-MSCs (5×10^5 cells), exhibited the most pronounced suppression of IL-1β levels among the treated groups ($p<0.05$). On the other hand, group K3 (924.52±176.38 pg/mL) showed no significant ($p>0.05$) difference in IL-1β levels compared to group K2 (931.94±205.80 pg/mL), suggesting that HA alone does not mitigate UVB-induced inflammation, as illustrated in Figure 3. These findings underscore the potential of H-MSC therapy to counteract UVB-induced inflammation by reducing IL-1β expression in skin tissue.

H-MSC administration inhibits NF-κB p65 transcription factor in rat skin with UVB-induced collagen loss

The expression of the NF-κB p65 transcription factor in skin tissue was analyzed using qRT-PCR. Results demonstrated a significant upregulation of NF-κB p65 expression in group K2 (2.46±0.50 a.u.) compared to group K1 (1.5±0.36 a.u.) ($p<0.05$). In contrast, the administration of H-MSCs in groups K4 (1.13±0.50 a.u.) and K5 (0.72±0.22 a.u.) effectively reduced NF-κB p65 expression levels compared to group K2 (2.46±0.50 a.u.) ($p<0.05$). The results also showed a reduction in p65 NF-κB expression in group K3 (1.08±0.48 a.u.), suggesting that HA also mitigates NF-κB p65. However, group K5 (0.72±0.22 a.u.), which received 5×10^5 H-MSCs, exhibited the lowest ($p<0.05$) levels of NF-κB p65 expression among all groups, as shown in Figure 4. These findings suggest that H-MSC treatment mitigates UVB-induced inflammatory signaling by suppressing the activation of the NF-κB p65 pathway, thereby offering a potential therapeutic strategy for preventing collagen loss.

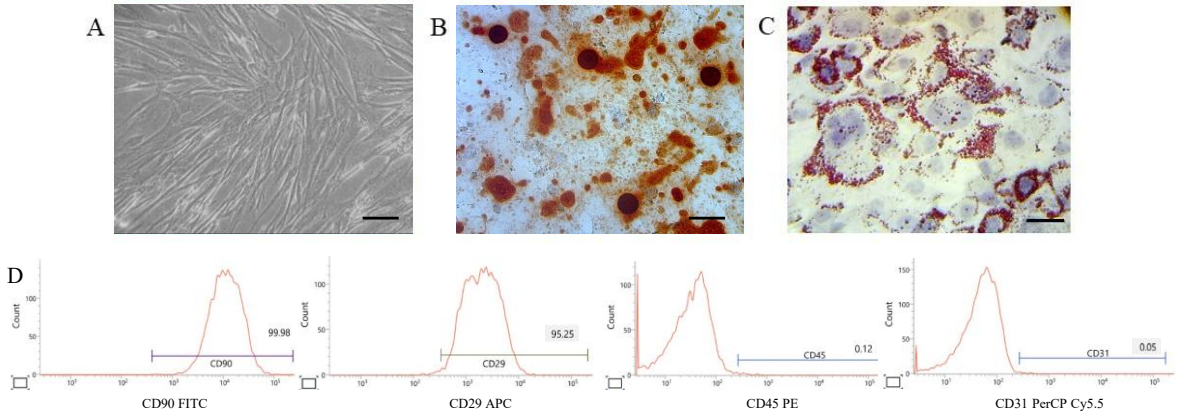

Figure 1. The characteristics of H-MSCs
H-MSCs at passage 5 exhibited spindle-shaped, fibroblast-like morphology (A). adipogenic (B) and osteogenic (C) differentiation was confirmed by oil red O and alizarin red staining (red coloration). Images were captured at 200× magnification. Immunophenotyping analysis showed high expression of CD90 (99.8%) and CD29 (95.3%) and low expression of CD45 (0.1%) and CD31 (0.1%), validating their mesenchymal origin

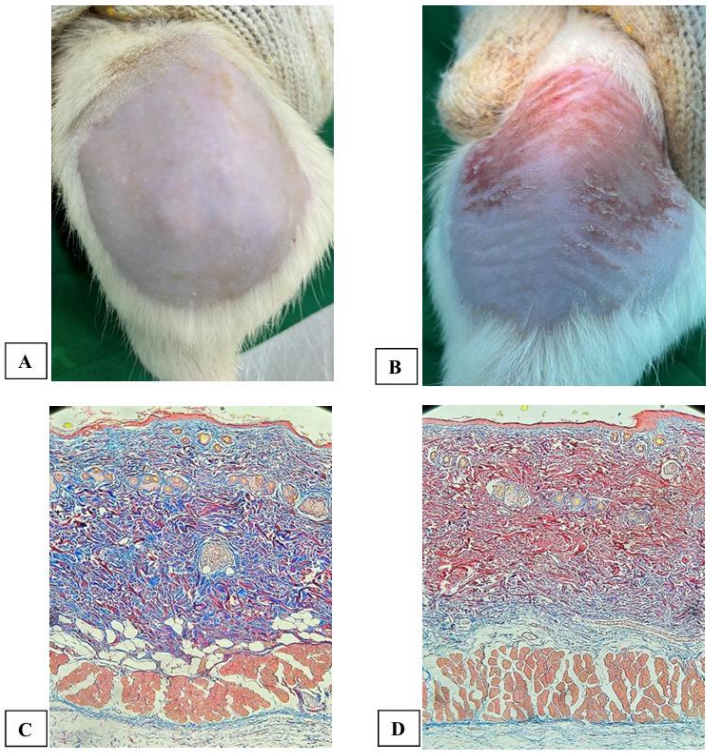

Figure 2. Validation of collagen loss
Macroscopic and histological validation of collagen degradation induced by UVB irradiation. Healthy rats showed smooth skin with no visible wrinkles (A), whereas UVB-irradiated rats without treatment exhibited prominent wrinkling (B). Collagen staining using Masson's Trichrome demonstrated higher collagen density (indicated by blue staining) in healthy rats (C) compared to a significant reduction in collagen content in UVB-irradiated rats without treatment (D). These findings confirm successful induction of collagen loss in the UVB-irradiated animal model.

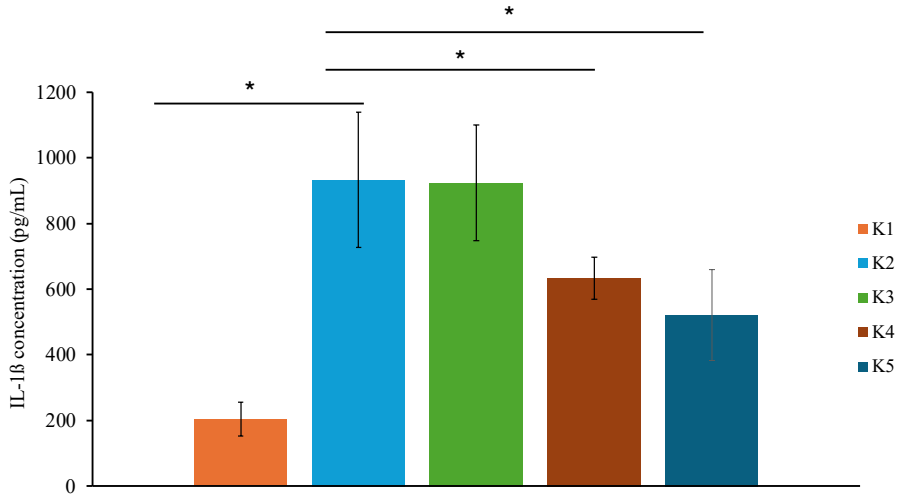

Figure 3. IL-1β level in skin tissue after 14 days intervention by treatment groups
IL-1β expression was significantly increased in the collagen loss group (K2: 931.94±205.80 pg/mL) compared to the healthy control group (K1: 203.99±51.76 pg/mL) ($p<0.05$). Treatment with HA in group K3 (924.52±176.38 pg/mL) did not result in a notable change in IL-1β expression compared to the collagen loss group (K2). However, the groups treated with H-MSCs (K4: 633.14±63.76 pg/mL and K5: 520.80 ± 137.81 pg/mL) showed significantly lower IL-1β levels compared to the collagen loss group (K2: 931.94±205.80 pg/mL) ($p<0.05$), with the lowest IL-1β expression observed in the group receiving the higher H-MSC dose (K5: 520.80±137.81 pg/mL). Data are presented as mean ± SD. *$p<0.05$. K1: healthy rat group; K2: collagen loss group; K3: collagen loss + HA treatment group; K4: collagen loss+2.5 × 10^5 H-MSC treatment group; K5: collagen loss + 5 × 10^5 H-MSC treatment group.

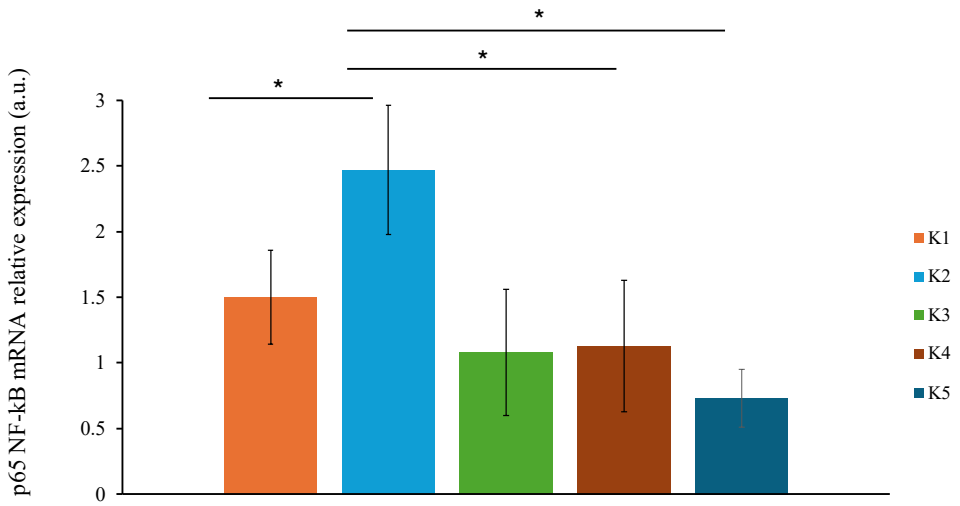

Figure 4. Gene expression of NF-κB p65 in skin tissue after 14 days intervention by treatment groups
NF-κB expression was significantly increased in the collagen loss group (K2: 2.46±0.50 a.u.) compared to the healthy control group (K1: 1.50±0.36 a.u.) ($p<0.05$). The groups treated with H-MSCs (K4: 1.13±0.50 a.u.) and K5: 0.72±0.22 a.u.) showed significantly lower NF-κB levels compared to the collagen loss group (K2: 2.46±0.50 a.u.) ($p<0.05$), with the lowest NF-κB expression observed in the group receiving the higher dose of H-MSCs (K5: 0.72±0.22 a.u..). Data are presented as mean ± SD. *$p<0.05$. K1: healthy rats group; K2: collagen loss group; K3: collagen loss + HA treatment group; K4: collagen loss+2.5 × 10^5 H-MSC treatment group; K5: collagen loss + 5 × 10^5 H-MSC treatment group. a.u : arbitrary units

DISCUSSION

Our findings demonstrated a significant reduction in NF-κB p65 expression and IL-1β levels across all treatment groups compared to the negative controls. Notably, the optimal reduction occurred in the group treated with a $5x10^5$ H-MSC dose in 200 µL NaCl. This aligns with a previous study showing that H-MSCs can decrease p65 expression in the hyperpigmented animal model.[18] Another study also reported that Wharton's jelly-derived MSCs decreased IL-1β gene expression in an Alzheimer's rat model.[21] These findings suggest that H-MSCs also possess the capacity to downregulate p65 and IL-1β expression in UVB-induced animal models.

UVB radiation is a major cause of collagen loss, often accompanied by excessive inflammatory responses.[22] The inflammatory response induced by UVB radiation is primarily driven by the activation of the NF-κB signaling pathway, particularly the p65 subunit in the epidermis, resulting in the secretion of various pro-inflammatory cytokines, such as IL-1β.[2] A previous study highlighted the critical role of NF-κB p65 in maintaining and expanding the inflammatory process in the skin, suggesting that modulation of this signaling pathway could attenuate UVB-induced inflammatory responses.[19]

The anti-inflammatory effect observed in this study could be attributed to the IL-10 secreted by the H-MSCs, which plays a crucial role in suppressing the inflammatory process associated with collagen loss, thereby downregulating NF-κB p65 expression and IL-1β production. This is in line with a previous study, which demonstrated that IL-10 suppresses inflammation via activation of intracellular proteins, such as suppressor of cytokine signaling 3 (SOCS3).[23] IL-10 binds to its receptor, initiating the signal transducer and activator of transcription 3 (STAT-3) pathway. STAT3 then translocates to the nucleus and induces the mRNA sequence for SOCS3, which subsequently inhibits several pro-inflammatory signaling pathways, including NF-κB p65. This attenuation of NF-κB p65 results in a decrease in the secretion of pro-inflammatory cytokines, including IL-1β.[24]

H-MSCs have demonstrated considerable potential in treating various forms of inflammation and promoting tissue regeneration.[8] Hypoxic preconditioning (culturing MSCs under low oxygen conditions) has emerged as a promising approach. Studies have shown that H-MSCs enhance the secretion of anti-inflammatory cytokines and growth factors, helping regulate inflammatory processes and accelerating wound healing.[9] H-MSCs are known to increase IL-10 production, when administered at low doses in cases of ischemia/reperfusion-induced lung injury.[10] IL-10 which plays an essential role in suppressing excessive immune responses and reducing pro-inflammatory cytokine production, including IL-1β and TNF-α.[10] By decreasing the levels of pro-inflammatory cytokines, H-MSCs help mitigate inflammation within tissues. Furthermore, H-MSCs have been shown to modulate the NF-κB pathway, a major pathway in the inflammatory response.[25] Activation of NF-κB p65 is associated with elevated pro-inflammatory cytokine expression. By modulating this pathway, H-MSCs can reduce NF-κB activation, thereby decreasing IL-1β and other pro-inflammatory cytokines.[16] Beyond their anti-inflammatory role, H-MSCs also promote collagen synthesis through the secretion of growth factors such as TGF-β.[18]

Our study underscores the clinical relevance of H-MSC-based therapies as novel and potentially safer alternatives for managing UVB-induced skin damage and inflammation-related collagen loss. By targeting key pro-inflammatory mediators and restoring extracellular matrix homeostasis, H-MSCs offer a regenerative strategy with broad therapeutic potential in dermatology and tissue repair.[17,26] Notably, while hyaluronic acid (HA) treatment alone did not attenuate inflammatory markers, H-MSC treatment significantly downregulated both p65 and IL-1β, indicating a superior anti-inflammatory profile and highlighting the therapeutic advantage of cell-based interventions over conventional topical agents.[7,27] While this study demonstrates the promising anti-inflammatory effects of H-MSCs in the rat collagen loss model, several limitations warrant consideration for future investigations. Although the study provides valuable insights into the anti-inflammatory effects of H-MSCs, the underlying molecular mechanisms, particularly how H-MSCs modulate other inflammatory pathways beyond NF-κB, remain unclear. Further research is needed to elucidate the full spectrum of cytokine interactions and cellular pathways involved in the H-MSCs anti-inflammatory and regenerative effects.

CONCLUSION

Our findings provide compelling evidence that subcutaneous injection of H-MSCs effectively mitigates inflammation in the rat collagen loss model by downregulating the expression of NF-κB p65 and reducing IL-1β levels. Our data underscore the therapeutic potential of H-MSCs as a targeted intervention for inflammatory disorders, particularly those associated with collagen depletion and impaired synthesis in the skin. The anti-inflammatory and regenerative capabilities of H-MSCs, especially in modulating key inflammatory pathways and promoting tissue repair, suggest that they could offer significant clinical benefits for conditions such as photoaging. Given the promising outcomes observed, further clinical investigations are necessary to explore the broader application of H-MSCs in the management of skin aging and other inflammatory skin diseases.

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Ethics approval

Institutional Research Bioethics of Universitas Islam Sultan Agung no 421/X/2024/Komisi Bioetik.

Competing interests

All the authors declare that there are no conflicts of interest.

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Author Contributions

Conceptualization: YF, AP, TS, ES, SI, and DTP; Data curation: YF; Formal analysis: Y.E; Funding acquisition: AP, TS, ES, SI and DTP; Investigation: YF; Methodology: YF, AP, TS, ES, SI, and DTP; Project administration: YF; Resources: AP, TS, ES, SI, and DTP; Software: YF; Supervision: AP; Validation: AP and TS. All authors have read and approved the published version of the manuscript.

Data Availability Statement

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration the Use of AI in Scientific Writing

This manuscript has benefited from the use of artificial intelligence (AI)-assisted tools to support the scientific writing process. OpenAI's ChatGPT was employed for language refinement, grammar correction, summarization, paraphrasing, and formatting of scientific content. All scientific ideas, experimental designs, analyses, interpretations, and conclusions presented in this work were conceived, conducted, and validated solely by the authors. The AI was not involved in the generation of original data, analysis, or critical scientific decision-making. The authors take full responsibility for the integrity and accuracy of the content presented.

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