Indonesian Journal of Biotechnology

VOLUME 30(4), 2025, 217-225 | RESEARCH ARTICLE

Anti-inflammatory properties of conditioned medium from human Wharton's jelly mesenchymal stem cells

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SUBMITTED 23 May 2025 **REVISED** 18 December 2025 **ACCEPTED** 19 December 2025

ABSTRACT Acute respiratory distress syndrome (ARDS) is a critical respiratory dysfunction triggered by intense inflammation, microvascular damage, and increased epithelial and pulmonary vascular permeability. Human Wharton's jelly mesenchymal stem cells (hWJMSCs) possess regenerative and anti-inflammatory activities through the cytokines, chemokines, and growth factor secretion. The development of anti-inflammatory agents derived from hWJMSCs has become one of the therapeutic solutions. Instead of direct cell use of hWJMSCs, their conditioned medium (CM) provides a cell-free approach that delivers bioactive factors while minimizing the risks associated with stem cell transplantation. This study aims to measure the levels of vascular endothelial growth factor-α (VEGF-α), epidermal growth factor-β (EGF-β), interleukin-10 (IL-10), and hepatocyte growth factor (HGF) in CM-hWJMSCs under non-starvation and starvation conditions (24, 48 and 72 hours) using ELISA. The anti-inflammatory potential of these factors was then analyzed through molecular docking with pro-inflammatory cytokines. VEGF-α, EGF-β, IL-10 and HGF levels were measured across all conditions. VEGF-α ranged from 2590.37 to 3613.92 ng/mg protein; EGF-β 347.01–504.43 ng/mg; IL-10 302.59–729.28 pg/mg; and HGF 1747.20–2903.52 ng/mg. The molecular docking revealed strong binding between VEGF-α, EGF-β, IL-10 and HGF with pro-inflammatory cytokines, namely IL-1β, IL-6 and TNF-α. VEGF-α had the strongest bond with TNF-α (–1162.3 kJ/mol), while EGF-β formed the most hydrophobic and hydrogen interactions. The findings suggest that CM-hWJMSCs, enriched with anti-inflammatory and regenerative cytokines, may serve as a promising candidate for modulating the inflammatory pathways involved in ARDS pathogenesis. Longer starvation increased the secretion of VEGF-α, EGF-β, IL-10 and HGF. These factors are known to promote angiogenesis, regulate immune responses, and protect against epithelial injury, thereby supporting the anti-inflammatory and regenerative potential of hWJMSCs-CM for ARDS therapy.

KEYWORDS Anti-inflammatory properties; ARDS; Conditioned medium; Growth factor; hWJMSCs

1. Introduction

Inflammation described as a normal physiological response to tissue injury caused by various events that initiate it (Matsuda et al. 2019), including lung inflammation, which leading to acute respiratory distress syndrome (ARDS) (Li and Ma 2020). ARDS is an excessive inflammatory response by the body against viral infections. This inflammatory reaction is typically triggered by a surge of pro-inflammatory cytokines, a phenomenon commonly referred to as an excessive cytokine release. The consequences of this uncontrolled inflammation can lead to the failure of multiple organs and death (Yao et al. 2022). The severity of ARDS is correlated with elevated pro-inflammatory cytokines levels for instance interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) (Liu et al. 2022). Severe inflammatory responses trigger cellular and molecular processes that help mitigate potential injury or infection. This protective mechanism facilitates the restoration of tissue balance and the excessive inflammation resolution (Eze et al. 2019). Various cytokines levels rise in serum, namely IL-10, granulocyte-colony stimulating factor (G-CSF), chemokines, and hepatocyte growth factor (HGF) (Perreau et al. 2021). HGF is a versatile anti-inflammatory cytokine that contributes to repairing lung tissue. It acts as a significant counter-regulatory factor in

the host immune response, modulating pro-inflammatory cytokines and then preventing pulmonary fibrosis (Perreau et al. 2021).

In general, various bioactive factors secreted by mesenchymal stem cells (MSCs) contribute to tissue restoration; for instance, cytokines, epidermal growth factor-β (EGF-β), and HGF. MSCs exhibit strong immunomodulatory capabilities, a natural affinity for injured and inflamed tissues, and can be easily harvested and isolated, making them promising resources for cell-based therapies (Zhuang et al. 2021). MSCs are also able to produce substances that promote angiogenesis, encourage cell proliferation, and decrease apoptosis. MSCs release growth factors and angiogenesis-supporting factors, including vascular endothelial growth factor-α (VEGF-α) (Ting and Dilley 2018).

Several studies state that the conditioned medium of MSCs (CM-MSCs) contains cytokines, growth factors, bioactive lipids, enzymes, and nucleic acids (Bogatcheva and Coleman 2019; Sagaradze et al. 2019; Widowati et al. 2016). These factors are secreted by mesenchymal stem cells, including human Wharton's jelly MSCs (hWJMSCs) (Sagaradze et al. 2019). The starvation condition refers to culturing hWJMSCs in a conditioned medium without fetal bovine serum (FBS), while the non-starvation condition includes FBS in the culture medium (Widowati et al. 2021). Previous studies showed that longer periods of starvation, in which hWJMSCs were cultured in serum-free medium, increased the secretion levels of interleukin-1 receptor antagonist (IL-1ra), antimicrobial peptide LL-37, fibroblast growth factor-7 (FGF-7), and indoleamine 2,3 dioxygenase (IDO) in hWJMSCs (Widowati et al. 2021). Hence, in this study, CM from hWJMSCs (CM-hWJMSCs) was collected to investigate other parameters related to anti-inflammation and ARDS therapy including the level of EGF-β, IL-10, VEGF-α, HGF in starvation, and non-starvation-cell conditions.

2. Materials and Methods

2.1. Conditioned medium of hWJMSCs preparation

The isolated hWJMSCs were acquired from Aretha Medika Utama Bandung, Indonesia. Cells have been characterized with specific antibodies, namely CD73 (APC), CD44 (PE), CD90 (FITC), and CD105 (PerCP-Cy5), from the BD Stemflow™ Human MSC Analysis Kit (BD Biosciences, 562245), and negative lineage (Widowati et al. 2019, 2021). hWJMSCs at passage 5 (P5) were utilized in this study. The 8×10^3 cells/cm^2 cells were cultured in Minimum Essential Medium (MEM-α) (L0475500, Biowest) administered with 10% FBS (S1810-500, Biowest), 1% Amphotericin B (Amp B) (L0009100, Biowest), Nanomycopulitin (LX16-100, Biowest), and 0.1% Gentamicin (15750060, Gibco) at 37 °C, 5% CO_2. Conditioned medium (CM) refers to the culture medium that has been used to grow hWJMSCs and subsequently contains the bioactive factors secreted by these cells. In contrast, non-CM refers to fresh culture medium that has not been exposed to cells. Once the cells reached approximately 80–90% confluency, as visually assessed under an inverted microscope based on surface coverage, the medium was collected as CM under non-starvation conditions. Meanwhile, CM under starvation conditions was collected from hWJMSCs cells grown on an FBS-free medium (after 80-90% confluency) (Widowati et al. 2021) for 24, 48 and 72 h. Subsequently, the collected medium (under non-starvation conditions and under starvation conditions) was centrifuged at 3,000×g for 4 min, then the supernatant was passed through a filter by a 0.22- µm; Ø 33 mm (TPP-99722) (Widowati et al. 2021).

2.2. Measurement of VEGF-α, EGF-β, IL-10, and HGF levels of CM-hWJMSCs

VEGF-α, EGF-β, IL-10, and HGF levels in the CM-hWJMSCs were assessed using the ELISA kit VEGF-α (Elabscience, E-EL-H0111), EGF-β (Elabscience, E-EL-H0059), IL-10 (Elabscience, E-EL-H6154), and HGF (Elabscience, E-EL-H0084). The assays were performed on the cell-free conditioned medium (CM), in accordance with the manufacturer's protocol. Conditioned medium generally contains proteins secreted by hWJMSCs, including cytokines, growth factors, and other soluble bioactive molecules.

2.3. Total protein assay

Bovine serum albumin (BSA; Sigma, A2153-100G) 2 mg was diluted in 1000 µL of ddH_2O to prepare a standard BSA solution, and then 10 variations of standard concentrations were prepared (1,500; 1,000; 750; 500; 250; 125; 25; 5; 1; and 0 µg/mL). Ten standard concentrations were prepared by serial dilution of the 2 mg/mL BSA stock solution using distilled water as the diluent. A total of 20 µL of each standard or sample solution was added to a 96-well plate, followed by 200 µL of QuickStart Bradford Dye Reagent 1× (Bio-Rad, 5000205), resulting in a 1:10 sample-to-reagent ratio. The mixture was gently mixed by pipetting and incubated at room temperature for 5 min. Absorbance was then measured at 595 nm using a Multiskan GO microplate reader (ThermoScientific) (Widowati et al. 2019; Noverina et al. 2019).

2.4. Statistical analysis

The data values were presented as mean and standard deviations from three experimental replicates. Data analysis was conducted using SPSS, while data visualization was conducted using GraphPad. For parametric data, analysis was conducted using ANOVA then post hoc Tukey HSD. Meanwhile, for non-parametric data, the Kruskal-Wallis test was performed then the Mann-Whitney test was utilized for analysis. A p value of < 0.05 was utilized to determine the significance.

2.5. Molecular docking

The anti-inflammatory potential of hWJMSCs was analyzed through molecular docking. The pro-inflammatory

protein sequence (IL-1β, IL-6, TNF-α) was acquired from the database on www.uniprot.org. The three sequences were formed into 3D structures using the Swiss Model webserver (www.swissmodel.expasy.org) to generate reliable protein conformations based on homology modeling. The sequences of IL-1β, IL-6, and TNF-α were obtained from Uniprot and visualized in the Swiss Model webserver with IDs P01375, P05231, and P01584, respectively. Molecular docking was used to analyze VEGF-α, EGF-β, IL-10, and HGF against pro-inflammatory proteins associated with ARDS, including IL-1β, IL-6, and TNF-α. The 3D structures of the proteins secreted by hWJMSCs, namely VEGF-α, EGF-β, IL-10, and HGF was acquired from the Research Collaboratory for Structural Bioinformatics (RCSB) in format Protein Data Bank (PDB) with IDs 4KZN, INQL, 8SVE, and 1BHT, respectively. The protein structure was then processed in Autodock 4.2 to remove native ligand and water, ensuring that the binding sites were accessible for docking. The protein–protein molecular docking simulation was done on Cluspro's webserver (www.cluspro.bu.edu) which is specifically designed for predicting protein interaction complexes. The Discovery Studio software was used to visualize the docking complexes and analyze hydrogen bond formation as well as hydrophobic interactions. While VEGF-α, EGF-β, IL-10, and HGF are classically recognized to interact with their respective physiological receptors, recent computational approaches allow protein–protein docking to be used in an exploratory manner to assess potential interaction interfaces at the structural level. Therefore, in this study, docking was applied to screen possible binding orientations between CM-derived proteins and pro-inflammatory mediators (IL-1β, IL-6, and TNF-α). This approach has been increasingly used to predict alternative binding surfaces that may provide preliminary mechanistic hypotheses for anti-inflammatory activity. Accordingly, the docking results in this study are interpreted as supportive in silico evidence, rather than confirmation of *in vivo* binding events.

3. Results and Discussion

3.1. The level of VEGF-α, EGF-β, IL-10, and HGF in hWJMSCs-CM

VEGF-α concentration in CM showed an increased trend along with the prolonged incubation time of 24-, 48-, and 72-h starvation. According to the result, the VEGF-α level reached the peak at 72 h compared to 24-, and 48-h starvation and under non-starvation conditions ($p < 0.05$) (Figure 1a, b). Figure 1, 48-, and 72-h starvation conditions. A significant increase in EGF-β levels was observed with prolonged starvation durations ($p < 0.05$). Notably, the EGF-β level in the CM under non-starvation conditions group was the lowest when compared to the groups subjected to 24-, 48-, and 72-h of starvation. The IL-10 levels for under non-starvation conditions, 24-, 48-, and 72-h starvation conditions were measured, showing a significant increase in IL-10 with longer starvation periods ($p < 0.05$). The 72-h starvation group exhibited the highest IL-10 levels compared to the other treatments (Figure 1e, f). The HGF levels across under non-starvation conditions, 24-, 48-, and 72-h starvation periods were evaluated, revealing a progressive increase in HGF concentrations with extended starvation durations ($p < 0.05$). The 72-h starvation period exhibited the highest HGF levels, while the under non-starvation conditions group displayed the lowest. These findings suggested that prolonged starvation contributed to elevated HGF levels (Figure 1g, h).

3.2. Molecular docking

Protein modeling scores for IL-1β, IL-6, and TNF-α were 100%, 90.71%, and 94.85%, respectively, based on the percentage identity between the query sequences and the selected templates used in Swiss-Model, with full sequence coverage. Figure 2 presents both the 3D structure and the Ramachandran plot of each modeled pro-inflammatory cytokine (IL-1β, IL-6, and TNF-α), used to assess structural quality prior to docking. The molecular docking results indicated strong binding interactions between VEGF-α, EGF-β, IL-10, and HGF with pro-inflammatory cytokines IL-1β, IL-6, and TNF-α (Figure 3). The binding affinity values, ranging from –970.1 to –1,166.5 kJ/mol, suggested a high binding strength. VEGF-α demonstrated the strongest interaction with TNF-α (–1162.3 kJ/mol). EGF-β formed the highest number of hydrogen bonds and hydrophobic interactions (Table 1). The numbers in Table 1 represent the total count of hydrogen bonds and hydrophobic interactions observed in each docked protein–protein complex. Although there is no universal cutoff for interaction strength, a higher number of these interactions generally correlates with greater binding stability. These findings suggested that these biomolecules may effectively inhibit inflammatory responses by stabilizing pro-inflammatory cytokines.

In the Ramachandran plot, the axes represent the φ (phi) and ψ (psi) backbone dihedral angles. Green regions indicate energetically allowed conformations, while white areas represent disallowed regions. Dots indicate individual residues: red (most favored regions), yellow (additional allowed), light green (generously allowed), and blue (disallowed). In addition to binding affinity measurements, the docking results also revealed consistent structural compatibility between all four hWJMSC-secreted proteins (VEGF-α, EGF-β, IL-10, HGF) and each of the pro-inflammatory cytokines. Although these interactions do not represent native receptor–ligand biology, the formation of multiple hydrogen bonds and hydrophobic contacts suggests that these proteins may form stable protein–protein complexes. These findings provide preliminary structural insight into potential anti-inflammatory mechanisms that extend beyond classical receptor signaling pathways.

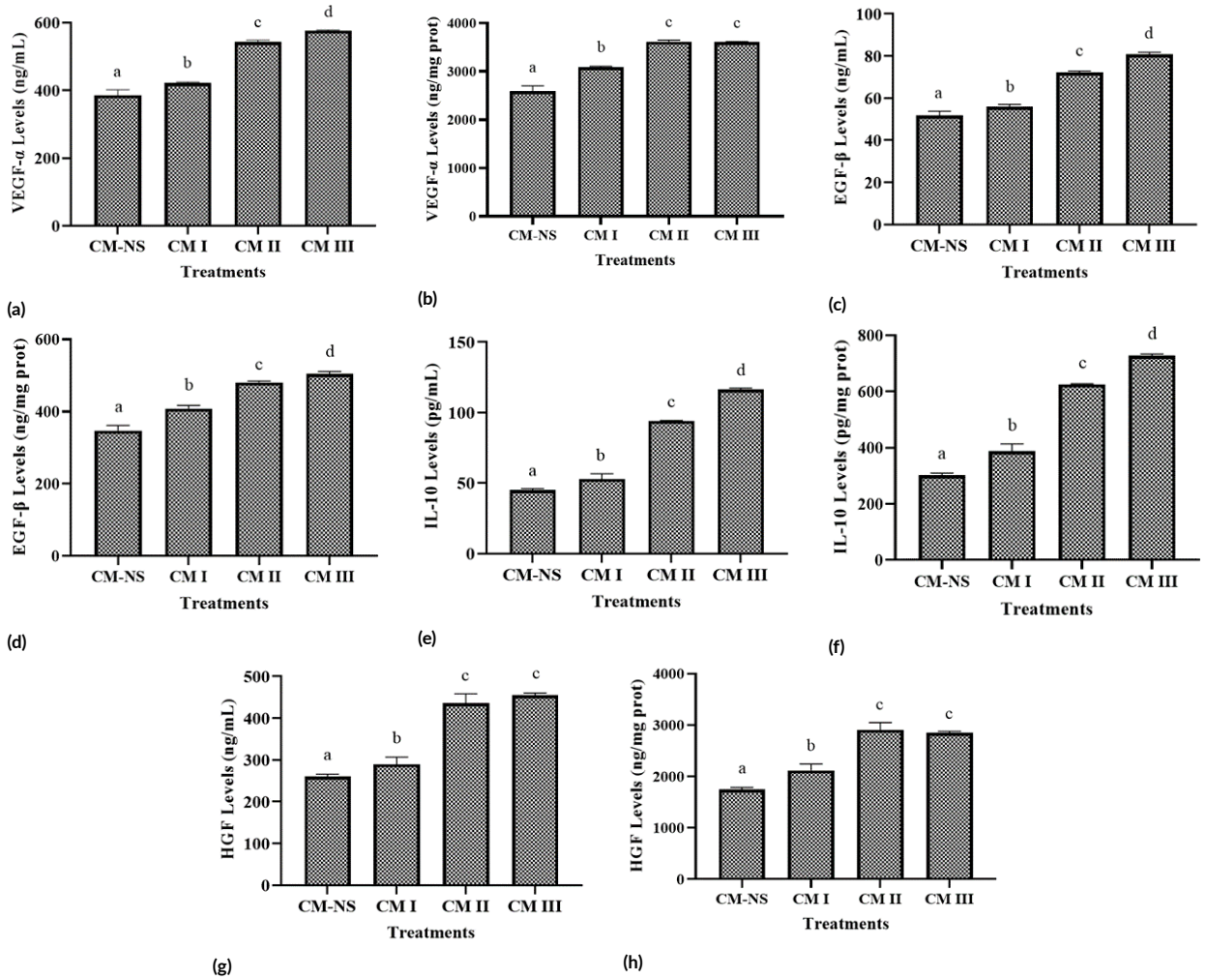

FIGURE 1 Effect of various starvation period treatment to VEGF-α, EGF-β, IL-10, and HGF level in CM-WJMSCs. (a) VEGF-α level of CM-WJMSCs (ng/mL), (b) VEGF-α level of CM-WJMSCs (ng/mg protein), (c) EGF-β level of CM-WJMSCs (ng/mL), (d) EGF-β level of CM-WJMSCs (ng/mg protein), (e) IL-10 level of CM-WJMSCs (ng/mL), (f) IL-10 level of CM-WJMSCs (ng/mg protein), (g) HGF level of CM-WJMSCs (ng/mL), (h) HGF level of CM-WJMSCs (ng/mg protein). Different letter indicates statistically significant differences in VEGF-α levels across starvation durations, analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U post hoc test ($p < 0.05$). Statistical comparisons for EGF-β, IL-10, and HGF were performed using one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). The treatment grouping: CM-NS (Conditioned medium under non-starvation conditions), CM I (Conditioned edium under starvation conditions 24-h), CM II (Conditioned medium under starvation conditions 48-h), CM III (Conditioned medium under starvation conditions 72-h).

3.3. Discussion

Mesenchymal stem cells (MSCs) are categorized as biologic agents with promising roles in immunomodulation, tissue preservation, and regeneration. Alongside cell-based treatments, there is growing interest in cell-free approaches utilizing MSC-secreted bioactive molecules. These cells are known to release various lipid mediators, RNA, proteins, and peptides (Bogatcheva and Coleman 2019). MSCs regulate tissue development and maintenance by producing several secretory factors (Gnecchi et al. 2016; Kim et al. 2019). The application of CM-MSCs can be a strategy for regenerative medicine because several CM-MSCs have been tested in various diseases with many demonstrating positive results (Sagaradze et al. 2019).

In this study, the longest starvation (72 h) had the highest levels of VEGF-α, EGF-β, IL-10, and HGF. This result confirmed that exposure of MSCs to hypoxia or prolonged starvation during conditioning led to an increase in cytokines, chemokines, and growth factors including VEGF-α (Hwang et al. 2020). A previous study stated that when cells experience insufficient oxygen and nutrient availability, the levels of other pro-angiogenic molecules increase (Jiménez-Valerio and Casanovas 2017). Moreover, pro-angiogenic factors namely VEGF-α, platelet-derived growth factor (PDGF), HGF, and angiopoietin-1 (ANG-1), which are released by MSCs, that trigger angiogenesis (Moradinasab et al. 2021). Genetic modification of MSCs can also be performed to enhance the secretion of other factors, regulate hypoxia-induced factor expression, or insert tissue-targeting peptides into the secreted extracellular vesicle membrane (Bogatcheva and Coleman 2019). Additionally, other studies have reported that tissue injury resulting from trauma disrupts nutrient flow, leading to cellular hypoxia and starvation. Due to disrupted

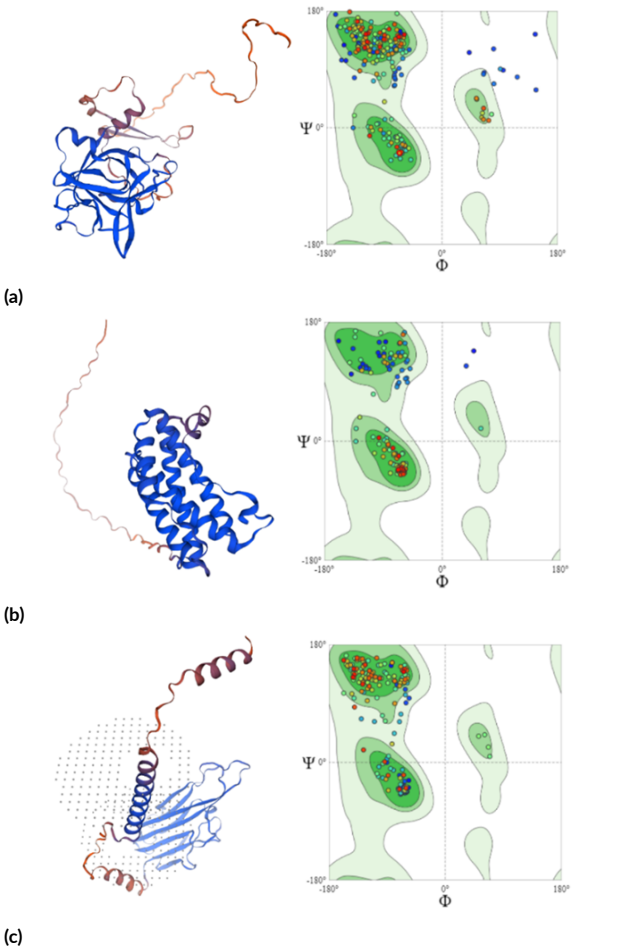

FIGURE 2 Three-dimensional models (left) and Ramachandran plots (right) of the modeled pro-inflammatory cytokines (a) IL-1β, (b) IL-6, (c) TNF-α. Each row represents one cytokine. In the 3D structure, the color gradient from blue to red indicates the N-terminal to C-terminal direction.

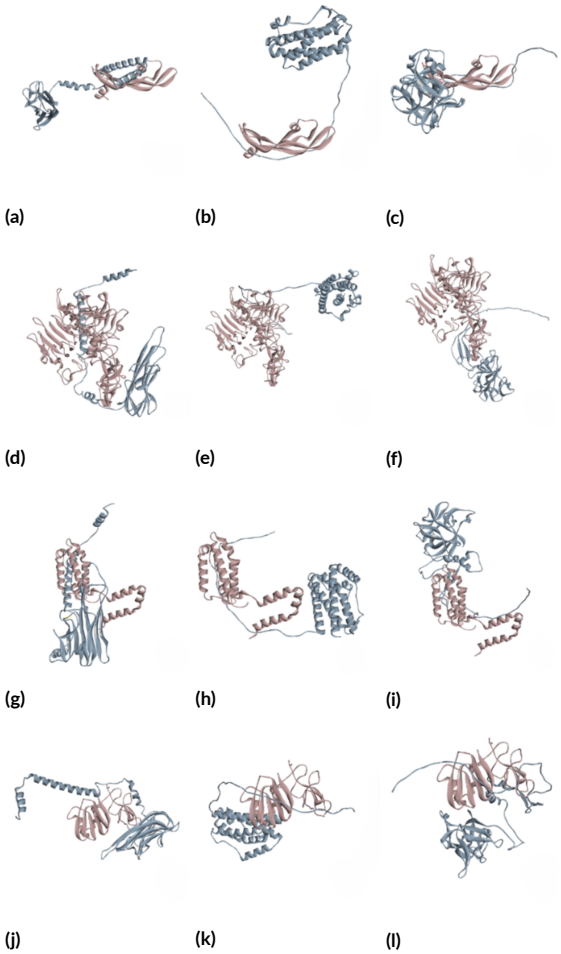

FIGURE 3 Molecular docking results of hWJMSCs with pro-inflammatory cytokines. (a) VEGF-α and TNF-α. (b) VEGF-α and IL-6. (c) VEGF-α and IL-1β. (d) EGF-β and TNF-α. (e) EGF-β and IL-6. (f) EGF-β and IL-1β. (g) IL-10 and TNF-α. (h) IL-10 and IL-6. (i) IL-10 and IL-1β. (j) HGF and TNF-α. (k) HGF and IL-6. (l) HGF and IL-1β. In each docking image, the hWJMSCs-secreted protein (VEGF-α, EGF-β, IL-10, and HGF) is shown in red, while the pro-inflammatory cytokine (TNF-α, IL-6, and IL-1β) is shown in blue.

nutrient flow, cells adapt their metabolism or initiate an adaptive response that promotes angiogenesis and tissue repair, leading to increased production of cytokines and chemokines (Püschel et al. 2020). From a physiological perspective, the use of CM-hWJMSCs *in vitro* provides a simplified model of the paracrine mechanisms underlying acute respiratory distress syndrome (ARDS). Although it does not fully replicate the complex lung microenvironment, CM offers insight into how secreted factors may modulate the excessive inflammation observed in ARDS (Yao et al. 2022; Moradinasab et al. 2021). These bioactive molecules including VEGF-α, HGF, EGF-β, and IL-10 are also reported to be elevated in patients with severe lung injury, suggesting that the CM model partially reflects the pathological cytokine and growth factor milieu during ARDS (Perreau et al. 2021).

As previously mentioned, MSCs produce VEGF-α, EGF-β, keratinocyte growth factor (KGF), HGF, and other growth factors that significantly contribute to tissue regeneration in injured lungs by reducing collagen accumulation and fibrosis. Through MSC secretion, VEGF and HGF can also repair and stabilize endothelial barrier function and regulate pulmonary capillary permeability in acute lung injury. HGF restores lung permeability by preventing epithelial cell apoptosis and regulating cell growth, morphogenesis, and motility through its interaction with the c-Met receptor. It is regarded as a key mediator in the MSCs therapeutic effects in ARDS (Loy et al. 2019).

MSCs perform their functions through paracrine activity, CM-MSCs reverse lung injury via KGF, which repairs injured epithelial cells by activating Na-K ATPase activity and anti-inflammatory cytokines such as matrix metallopeptidase-9 (MMP-9), IL-1α, and granulocyte macrophage-colony stimulating factor (GM-CSF) (Yao et al. 2022). Overexpression of several factors released by MSCs, namely EGF-β, angiogenin-1, PDGF-β, and FGF-β, facilitates lung repair and promotes cell proliferation (Wang et al. 2018). Overexpression of Chemokine Receptor-4 (CXCR4), ANG-1, Angiotensin-converting enzyme-2 (ACE-2), HGF, and KGF attenuates endotoxin-induced lung injury by reducing collagen depo-

TABLE 1 Hydrogen bonds and hydrophobic interactions in docked protein-protein complexes.

	Binding Affinity and Interaction	TNF-α	IL-6	IL-1β
VEGF-α	Binding affinity (kJ/mol)	−1,162.3	−1,096.6	−1,015.2
	Hydrogen bond	107	110	120
	Hydrophobic interaction	58	55	52
EGF-β	Binding affinity (kJ/mol)	−1,025.3	−970.1	−1,074.4
	Hydrogen bond	712	711	711
	Hydrophobic interaction	253	249	261
IL-10	Binding affinity (kJ/mol)	−1,131.0	−1,109.5	−1,037.3
	Hydrogen bond	236	237	265
	Hydrophobic interaction	62	63	61
HGF	Binding affinity (kJ/mol)	−1,148.5	−1,021.2	−1,166.5
	Hydrogen bond	251	236	258
	Hydrophobic interaction	102	101	99

sition, fibrosis, and edema, partly through enhanced anti-inflammatory and chemotactic effects (Wang et al. 2021).

The proteins secreted by hWJMSCs, such as VEGF-α, EGF-β, IL-10, and HGF, are known to play crucial roles in regulating inflammation and tissue repair. Although molecular docking is a predictive tool, the interactions identified in this study may reflect biologically relevant mechanisms. For example, IL-10 can directly bind to its receptor and modulate downstream cytokine production, while VEGF-α and HGF can influence vascular permeability and immune cell migration. The docking results suggest that these secreted factors may interact with pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), potentially interfering with their signaling pathways and contributing to the resolution of inflammation. These predicted interactions, if occurring naturally, could represent one mechanism by which hWJMSCs exert their immunomodulatory effects in conditions such as ARDS.

Molecular docking analysis revealed interactions between proteins. Hydrogen bonds are crucial for stabilizing the complex, determining ligand binding orientation, and optimizing hydrophobic interactions (Varma et al. 2010). Hydrophobic interactions are essential for enhancing binding affinity by guiding the ligand into the binding pocket (Varma et al. 2010). A study by Ferenczy and Kellermayer (2022) found that hydrogen bonds provide greater mechanical resistance than hydrophobic interactions. This study found that VEGF-α, EGF-β, IL-10, and HGF formed hydrogen bonds and hydrophobic interactions with each pro-inflammatory protein. These interactions are important indicators of binding affinity in molecular docking and suggest a potential for inhibitory activity. However, strong binding alone does not directly confirm functional inhibition, and further experimental validation is required. In support of this, the binding energy values obtained in our docking simulations ranged from –970.1 to –1,166.5 kJ/mol (Table 1), indicating relatively strong predicted interactions compared with typical protein–protein docking scores reported in the literature (Varma et al. 2010). However, as molecular docking provides only computational predictions, functional validation *in vitro* or *in vivo* is required to confirm whether these interactions result in actual inhibitory activity (Ferenczy and Kellermayer 2022).

The protein–protein interactions observed in this study through molecular docking support these biological activities by indicating the potential of VEGF-α, HGF, EGF-β, and IL-10 to directly bind and modulate pro-inflammatory cytokines. These interactions may contribute to their known anti-inflammatory and regenerative functions in lung injury, particularly in the context of ARDS. Although the proteins secreted by hWJMSCs normally signal through their respective native receptors, the docking results demonstrated strong structural interactions with IL-1β, IL-6, and TNF-α. These data should be interpreted as exploratory rather than physiological; however, they indicate that these proteins may potentially stabilize or interact with pro-inflammatory cytokines at a structural level. This could support an additional anti-inflammatory contribution beyond canonical receptor-mediated pathways. These insights align with current views that hWJMSCs-derived molecules exert complex immunomodulatory effects and may interact through multiple complementary mechanisms. Further biological validation will be required to confirm whether such interactions occur *in vivo*.

The mechanism of CM-hWJMSCs as anti-inflammatory agent in ARDS shown in Figure 4. ARDS is often driven by an excessive immune response that results in lung tissue damage and persistent inflammation. This process is largely driven by the pro-inflammatory cytokine overproductions, namely IL-1β, IL-6, and TGF-β, which are regulated by the NF-κB signaling pathway. The NF-κB activation further amplifies the inflammatory cascade, worsening lung injury and disease severity. CM-hWJMSCs offers a potential therapeutic approach to mitigate ARDS. CM-hWJMSCs consists of various bioactive factors, including VEGF-α, EGF-β, HGF, and IL-10, which exhibit anti-inflammatory and regenerative properties. These factors work collectively to inhibit

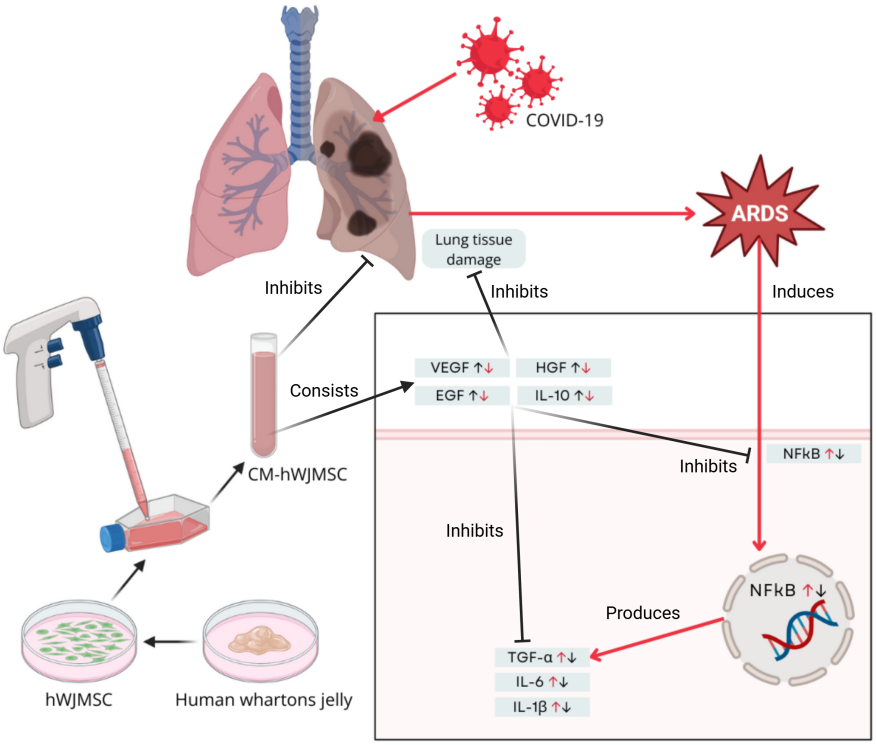

FIGURE 4 Proposed mechanism of CM-hWJMSCs in ARDS treatment.

NF-κB activation, suppress pro-inflammatory cytokines, and promote tissue repair. While the present study used an *in vitro* model, it is important to note that conditioned medium does not fully replicate the complexity of the lung microenvironment in ARDS, where multiple immune and structural cell types interact. Nevertheless, the bioactive factors identified in CM-hWJMSCs, such as VEGF-α, HGF, EGF-β, and IL-10, have also been reported to be elevated in patients with severe lung injury, suggesting partial relevance to the human condition. Thus, CM-hWJMSCs provide a useful model for studying paracrine mechanisms and may serve as a potential candidate for ARDS therapy, although further *in vivo* and clinical validation is required.

ARDS develops through excessive inflammatory signaling pathways that amplify lung injury and cytokine release. CM-hWJMSCs contain anti-inflammatory and regenerative factors that can reduce pro-inflammatory cytokine production and inhibit NF-κB activation, thereby helping to mitigate lung tissue damage and the progression of ARDS.

This study has several limitations. Although conditioned medium showed increased levels of VEGF-α, EGF-β, IL-10, and HGF, the individual contribution of each factor to the overall anti-inflammatory effect was not directly verified. Molecular docking results also represent computational predictions rather than biological confirmation and therefore cannot fully reflect receptor-mediated signaling or *in vivo* cytokine dynamics. In addition, mechanistic interpretation was not supported by pathway-based analysis. Future studies integrating network pharmacology, pathway profiling, and *in vivo* ARDS models will be necessary to validate molecular interactions and strengthen translational relevance.

4. Conclusions

Longer starvation periods in hWJMSCs increased the secretion of HGF, EGF-β, VEGF-α, and IL-10. These secreted proteins were then analyzed through molecular docking, which revealed their potential interactions with pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. This suggested that the conditioned medium of hWJMSCs has anti-inflammatory potential and may serve as a therapeutic candidate for ARDS and other inflammation-related conditions. As a preliminary study, the results of this study are expected to add information for further research and further studies for this research need to be carried out to prove this.

Acknowledgments

We acknowledge and thank you for grant, method and laboratory facilities from Aretha Medika Utama, Bandung, Indonesia.

Authors' contributions

RA, WW designed the study. RA, WW, RFG, HSWK carried out the laboratory work. RA, RFG, HSWK, DNT, DSH analyzed the data. RA, WW, DNT, DSH, MEG,

NM wrote the manuscript. All authors have reviewed and given their consent to the final manuscript.

Competing interests

The authors declare no competing interests.

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