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Cathelicidin in Mesenchymal Stem Cell Conditioned Medium Inhibit Pro-Inflammatory Cytokine and Osteoclastogenesis: A Bioinformatic Approach

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ABSTRACT

Mesenchymal stem cell conditioned mediun (MSC-CM) possessed abundant beneficial active compounds such as Cathelicidin or LL-37. Cathelicidin is antimicrobial peptide that may inhibit the microbial pathogen, pro-inflammatory cytokine and osteoclastogenesis. Cathelicidin may potential for drug development to treat osteolysis due to excessive pro-inflammatory cytokines induced by endotoxin. The aim of this study is to investigate MSCs-CM active compound namely Cathelicidin (LL-37) effect binding to tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), Receptor activator of nuclear factor κB (RANK), and tumor necrosis factor receptor 1 (TNFR1), interleukin- 1 receptor complex type 1 (IL-1RI), receptor activator complex of nuclear factor kappa-B ligand and osteoprotegerin (RANKL / OPG) by means of bioinformatics approach, *in silico* study. Sample preparation of target protein from RCSB PDB database. Then, the sample went through molecular docking method (rigid-body docking). Last, the sample visualize as 3D structure using PyMol software. The molecular docking simulation results showed that the binding condition with the cathelicidin TNFR_Cathelicidin complex at Rank 1 has the lowest binding energy with a global score of -83.94 kcal/mol, the IL1βR_ Cathelicidin complex at Rank 1 has the lowest binding energy with a global score of -8.43 kcal/mol. The RANK / TNFRSF11A_Cathelicidin complex in Rank 1 has the lowest binding energy with a global score of -21.67 kcal/mol. Ligand-receptor binding of Cathelicidin demonstrated by molecular docking inhibits various pro-inflammatory cytokines that may

inhibit osteoclastogenesis *in silico*.

1. INTRODUCTION

Rheumatoid arthritis, periodontal disease, and aseptic periprosthetic osteolysis are examples of bone diseases that can affect any part of the body. Bone disease is caused by chronic inflammation and characterized by bone loss around the joints and affected teeth due to increased osteoclastic bone resorption [1]. The prevalence of bone disease in the world is 1.5 million people every year, bone diseases such as those mentioned above if left untreated can lead to more severe conditions such as fractures. Epidemiological data show that in Singapore and Hong Kong, the hip fracture rate by age is 400 to 500 fractures per 100,000 women, close to the rate in the Caucasian population. In Malaysia and Thailand, the prevalence of fractures is 4-5% of the population. The risk of fracture increases with age in both men and women, as bones become more brittle and the risk of falling increases. Fractures are the most common musculoskeletal conditions that require hospitalization, this condition will also affect a person's activities so that if an individual experiences a fracture it will affect their health-related quality of life (HRQL) [2], [3].

Bone damage can be caused by congenital abnormalities and inflammation, clinical conditions that can trigger bone disease including Paget's disease, osteoporosis, and secondary bone changes caused by cancer, such as occurs in myeloma and metastases from breast cancer. Bone disease is often inherited genetically, for example in osteopetrosis and in pycnodysostosis due to cathepsin K deficiency. Bone disease can also be caused by inflammation such as in rheumatoid arthritis, periodontitis, when inflammation occurs the body will respond by releasing pro-inflammatory cytokines, for example TNF- a via its receptors results in increased transcription and activation of Nuclear Factor Kappa Beta (NF-kB), c-Fos and Nuclear Factor Associated T-cell 1 (NFATc1). tumor necrosis factor alpha (TNF-α) will induce osteoclast formation by activating its receptor on osteoclast progenitors, when osteoclasts bind to its receptor, Receptor activator of nuclear factor κB (RANK), bone resorption will occur [4].

Various kinds of therapeutic efforts for bone damage have been carried out, including treatment using antibiotics, combination antibiotics, surgical therapy, phyto-therapy using compounds obtained from herbal plants. In recent years, there has been an increase in studies and studies reporting the use of mesenchymal stem cells as an effort to treat bone damage. has been the focus of research efforts to exploit its therapeutic potential because MSCs have regenerative and immunoregulatory properties [5]. Despite these promising results, the findings obtained in the experimental and clinical studies that have been conducted indicate several limitations that must be overcome for the safe and efficient clinical use of Mesenchymal Stem Cells (MSCs). Safety issues regarding the undesired differentiation of transplanted MSCs are still a matter of debate, especially in the long-term follow-up in terms of manufacture, application, and cost. MSCs Conditioned Medium (MSCs-CM) is thought to provide advantages and potential for therapeutic applications over MSCs-based therapy. MSCs-CM as a cell-free therapy have advantages in storage, handling, product shelf life and potential as ready-to-use biologic products. Requirements, controls, regulations and arrangements for production and quality control are indispensable to establish the safety and therapeutic efficacy profile of the application of secretome MSCs. In addition to the immunomodulatory effects, secretome MSCs have a direct antibacterial effect, via LL-37 and the activity of the secreted peptides. LL-37

has antimicrobial properties to kill different microorganisms with the ability to prevent the immunostimulating effect of bacterial wall molecules such as lipopolysaccharides [6]. Thus, the aim of this study is to investigate MSCs-CM active compound namely Cathelicidin (LL-37) effect binding to TNF-α, Interleukin 1 beta (IL-1β), RANK, and tumor necrosis factor receptor 1 (TNFR1), interleukin- 1 receptor complex type 1 (IL-1RI), receptor activator complex of nuclear factor kappa-B ligand and osteoprotegerin (RANKL / OPG) by means of bioinformatics approach, *in silico* study.

2. Materials and methods

2.1 Sample Preparation

The ligand molecules in this study consisted of tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), Receptor activator of nuclear factor κB (RANK), and cathelicidin while tumor necrosis factor receptor 1 (TNFR1), interleukin- 1 receptor complex type 1 (IL-1RI), receptor activator complex of nuclear factor kappa-B ligand and osteoprotegerin (RANKL / OPG) as target proteins. The ligand and target protein samples were obtained from the RCSB PDB database (https://www.rcsb.org/), then downloaded in the protein databank (pdb) format.7 Samples were sterilized from contaminants consisting of native ligands and water molecules in PyMol software which aims to increase the effectiveness of docking analysis results [7- 9].

2.2 Protein Docking

Molecular docking is a simulation of the binding process of a molecule to a target through the help of computational software and aims to identify the interaction patterns of the complexes that are formed [10]. This study uses the docking proteins method, namely rigid-body docking, which aims to observe the formation of stable molecular complexes [11]. Webserver PatchDock (https://bioinfo3d.cs.tau.ac.il /PatchDock/index.html) & FireDock (http://bioinfo3d.cs.tau.ac.il/FireDock/) were used in this study to perform the simulation docking proteins. The docking result calculation refers to the total score of the low energy balance score [12], [13].

2.3 3D Structure Visualization

The docking simulation results are displayed through the PyMol software with structural selection and staining methods. Structural selection is the process of selecting target molecules to obtain specific structures such as cartoons, surfaces, sticks, ribbons, etc., while staining is selected to produce a representative appearance based on specific color differences [14].

3. Results

3.1 Ligand-receptor Binding Simulation & Cathelicidin Inhibitor Ability

The molecular docking simulation results show that under normal conditions the TNFα_TNFR complex at Rank 1 has the lowest binding energy with a global score of -103.54 kcal/mol, the Van der Waals interaction energy consists of attractive -40.80 kcal/mol and 20.20. kcal/mol for repulsive, and a hydrogen bond energy of -2.22 kcal/mol, the IL-β1_IL1βR complex at Rank 1 has the lowest binding energy with a global score of -23.85 kcal/mol, the Van der Waals interaction energy consists of attractive -24.24 kcal/mol and 11.45 kcal/mol for repulsive, and hydrogen bond energy of -1.77 kcal/mol, the RANKL / OPG_RANK / TNFRSF11A complex in Rank 1 has the lowest binding energy with a global score of - 27.78 kcal/mol, the interaction energy of Van deer Waals consists of attractive -14.65 kcal/mol and 15.86 kcal/mol, and hydrogen bond energies of -4.03 (Table 1).

The molecular docking simulation results show that in the binding condition with the cathelicidin

TNFR_Cathelicidin complex at Rank 1 has the lowest binding energy with a global score of -83.94 kcal/mol, the Van der Waals interaction energy consists of attractive -28.26 kcal/mol and 16,05 kcal/mol for repulsive, and a hydrogen bond energy of -2.22 kcal/mol, the IL1βR_ Cathelicidin complex at Rank 1 has the lowest binding energy with a global score of -8.43 kcal/mol, the interaction energy of Van deer Waals consists of for attractive -11.08 kcal/mol and 8.94 kcal/mol for repulsive, and hydrogen bond energy of -0.91 kcal/mol, the RANK / TNFRSF11A_Cathelicidin complex in Rank 1 has the lowest binding energy with a global score of -21,67 kcal/mol, the Van der Waals interaction energy consists of attractive -15.28 kcal/mol and 2.44 kcal/mol, and hydrogen bond energies of -0.76 (Table 2).

Rank 1 is chosen as the best position among the three ranks, this is a reference for comparing the binding energy level produced under normal conditions in the presence of cathelicidin. Based on the comparison of molecular docking data between normal conditions and the presence of a cathelicidin inhibitor, there is a difference in the binding energy formed by several types of weak bonds, the presence of the inhibitor can produce a decrease in the energy score, indicating that cathelicidin is possible to influence the target receptor activity through inhibition of the formation of the ligand-receptor complex under normal condition. The 3D structure of molecular docking visualization was carried out in PyMol software with the display of spheres, cartoons, and sticks (Figure 1).

Table 1. The result of ligand-receptor binding docking simulation under normal conditions

Molecular Complex	**Rank**	**Global Energy**	**Van deer Waals**		**Hydrogen Bond**
			Attractive	**Repulsive**	
TNFα_TNFR	1	-103.54	-40.80	20.20	-2.22
	2	-100.40	-41.77	23.39	-1.32
	3	-59.15	-24.19	12.93	-0.70
IL-1β_IL1βR	1	-23.85	-24.24	11.45	-1.77
	2	-20.84	-27.25	15.25	-3.69
	3	-14.31	-14.92	6.25	-1.06
RANKL/OPG_RANK/TNFRSF11A	1	-27.78	-14.65	15.86	-4.03
	2	-21.49	-10.42	21.34	-0.86

	3	-3.89	-27.24	22.00	-6.00

Table 2. The result of receptor binding docking simulation with inhibitors

Complex	**Rank**	**Global Energy**	**Van deer Waals**		**Hydrogen Bond**
			Attractive	**Repulsive**	
TNFR_Cathelicidin	1	-83.94	-28.26	16.05	-0.46
	2	-64.74	-23.91	9.53	-0.70
	3	-36.52	-18.92	8.98	0.00
IL1βR_ Cathelicidin	1	-8.43	-11.08	8.94	-0.91
	2	-2.92	-3.10	0.52	-0.63
	3	2.57	-5.93	1.79	0.00
RANK/TNFRSF11A_ Cathelicidin	1	-21.67	-15.28	2.44	-0.76
	2	-20.94	-22.89	15.19	-2.35
	3	-4.70	-30.55	20.74	-1.78

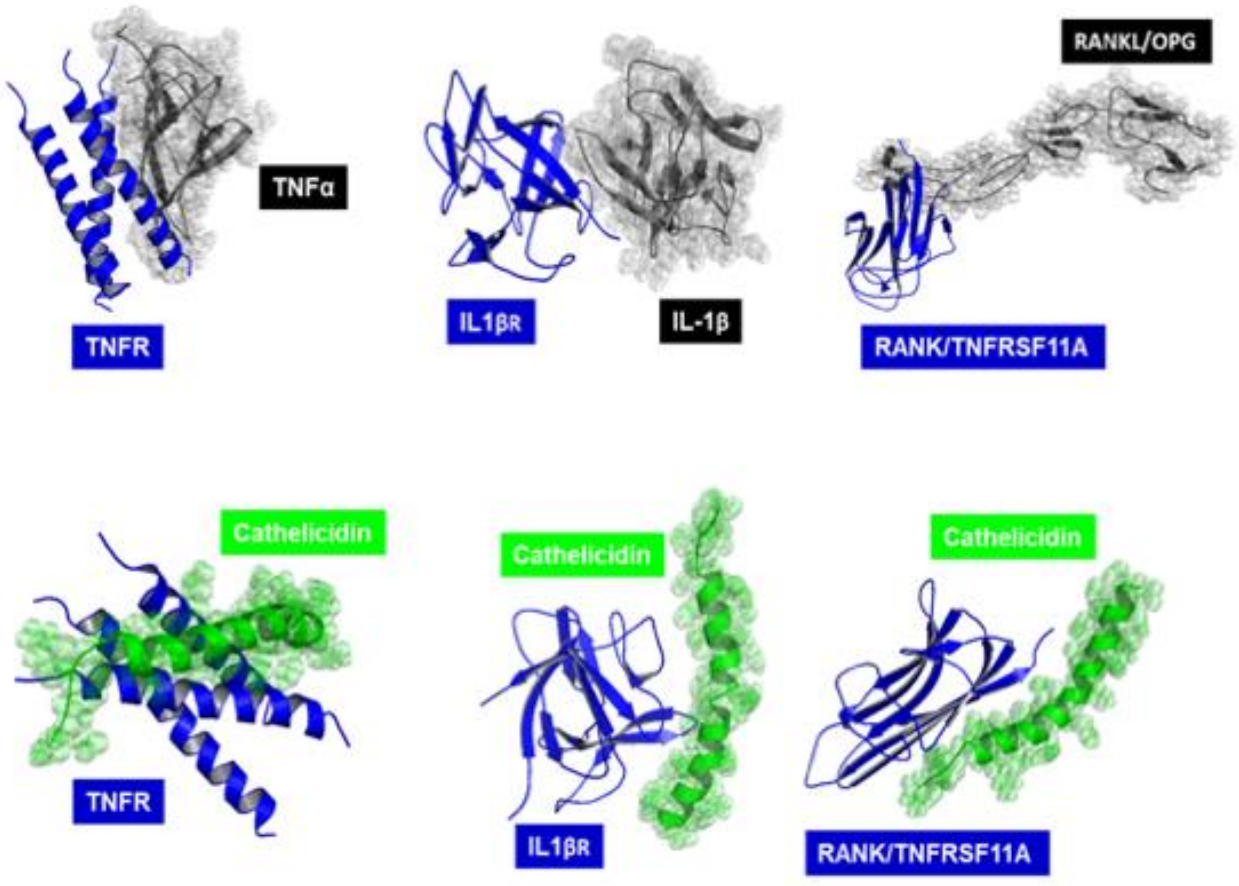

Figure 1. 3D molecular visualization of docking result.

4. Discussion

Cathelicidins are micropeptides produced by various immune cells which possess antimicrobial and immunomodulatory properties [15]. A plethora of Cathelicidins had been founded, which one of those is LL-37 is known to be released from macrophages, neutrophils, and various epithelial cells [16], [17]. Cathelicidins were previously only known as antimicrobial peptides) alongside defensins. It acts as antimicrobial agents by penetrating to biofilm, inhibiting bacterial attachment thus decreasing biofilm formation. Moreover, LL-37 was found to be a potent anti-microbial agent to S. aureus biofilm [18].

Meanwhile, findings on cathelicidins immense anti-inflammatory abilities are emerging. It exerts its anti-inflammatory capacity through induction of chemotaxis, immune cells activity, and angiogenesis [15]. Cathelicidins induces cells activities through binding with various G protein-coupled receptors such as MrgX2 and FPR2 to promotes Ca2+ intracellular signalling, thus leading to secretion of various cytokines and chemokines [19]. It was also founded that cathelicidins binds to TLRs such as TLR-2 and TLR-4 thus neutralizing LPS which leading to inhibiton NF-κB-mediated inflammatory genes expressions [19- 21]. Cathelicidins were also found to promote bone forming by inducing osteocalcin, osteonectin, bone sialoprotein and osteopontin expressions [22]. LPS neutralization of Cathelicidins is also potential to inhibit RANKL-RANK binding, thus leading to diminished osteoclastogenesis [19].

This study demonstrated the immunomodulatory mechanism of Cathelicidins by binding to TNF receptors (TNFRs) and IL-1β receptors (IL-1βRs), thus leading to reduced activity of both TNF-α and IL-1β in immune

cells. Molecular docking shows reduced binding affinity through reduced Van der Waals interaction energy and hydrogen bond of Cathelicidins-TNFR, cathelicidines-IL1βR and RANK/TNFRSF11A-Cathelicidin compared to the ones not inhibited by the AMPs. This would corroborate previous findings to anti-inflammation and bone forming properties of Cathelicidins. Study done by [23] shows that cathelicidins significantly reduced LPS-stimulated proinflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α in a dose dependent manner [23]. This can be caused by LL-37 action of inhibiting NF-κB phosphorylation, leading to reduced TNF-α expressions [24]. Cathelicidins are also found to be secreted by mesenchymal stem cells (MSCs), thus leading to notion of immense anti-bacterial and anti-inflammatory properties of MSCs secretomes [25], [26]. It was also found that these anti-inflammatory activities leading to increased bone formation through promoted osteogenic genes and diminished osteoclastic genes in the inflammatory microenvironment [19], [22]. Thus, utilization of MSCs-CM especially cathelicidins are promising to be a potent osteo-immunomodulatory agent in various bone inflammatory diseases.

5. Conclusions

Ligand-receptor binding of cathelicidins demonstrated by molecular docking inhibits various proinflammatory cytokines and osteoclastogenesis that may lead to bone regeneration escalation.

6. Conflict of Interest

The authors declare that there is no conflict of interest in this study.

7. Acknowledgement

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