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In Silico Approach for Pro-inflammatory Protein Interleukin 1β and Interleukin-1 Receptor Antagonist Protein Docking as Potential Therapy for COVID-19 Disease

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Abstract

BACKGROUND: Interleukin-1 receptor antagonist (IL-1Ra) also known as Anakinra is a receptor antagonist of IL-1 especially IL-1β. IL-1β increased in infected COVID-19 patient groups. This study aimed that the IL-1Ra contained in Conditioned Medium Wharton's Jelly Mesenchymal Stem Cells (CM-WJMSCs) has the potential to inhibit IL-1β which is one of the cytokine storms that occur in COVID patients through an *in --silico* approach.

AIM: This study aims to determine the effect of *in silico* approach pro-inflammatory protein interleukin 1β (IL-1 β) and IL-1Ra protein as cytokine WJ-MSCs for potential treatment of COVID-19 disease.

METHODS: 3D structure using the homology modeling method on Swiss Model web-server. Molecular docking was performed to analyze the binding mode of the IL-1β related to COVID-19 with IL-1Ra and the docking results were fixed using FireDock web-server.

RESULTS: These results of the docking of proteins between IL-1β and the CM-WJMSCs component, namely IL-1Ra showed that IL-1Ra has criteria for docking on IL-1β such as the good score for QMEAN, good CscoreLB, and BS-score results, and the lowest energy obtained was −585.1 KJ/mol. It can be predicted that IL-1Ra can inhibit IL-1β which causes cytokine storms in COVID-19 patients.

CONCLUSION: Based on the result, CM-WJMSC has potential treatment on the severity of COVID-19 infection.

Introduction

The cytokine network is self-regulating through the action of opposite cytokines, the release of soluble cytokine receptors, and the production of cytokine antagonists binding to receptors. Interleukin-1 receptor antagonist (IL-1Ra) is the first naturally occurring receptor antagonist described for any cytokine or hormone-like molecule. IL-1Ra has emphasized the biochemical properties of this unique cytokine, from its production and characterization of effects *in vitro* and *in vivo* [1]. IL-1Ra (MW ~17.6 kDa) is a receptor antagonist of IL-1, can competitively bind with IL-1R1 thereby blocking cell activation by the cytokine [2]. IL-1Ra have alternative name called Anakinra. Anakinra is a protein that differs from the sequence of Interleukin 1 receptor antagonist by one methionine added to its N-terminus also differs from the human protein in that it is not glycosylated, as it is manufactured in *Escherichia coli*. Anakinra has been approved by Food and Drug Administration (FDA) in 2001 [3].

Stem cells are immature tissue precursor cells that are able to self-renew, have the ability to form clonal cell populations, and differentiate into multiple cell [4], [5]. MSCs definitely secrete a wide range of factors, such as growth factors, cytokines, chemokines, and immunomodulatory molecules, which can promote neurogenesis and angiogenesis, or prevent pro-inflammatory reactions, and apoptosis. MSC-sourced secretome, such as conditioned medium (CM), is more economical and more practical for clinical application since it avoids invasive cell collection procedures [6]. CM-MSCs contain anti-inflammatory cytokines present are tumor necrosis factor β1 (TGFβ1), interleukin (IL) 13, IL10, IL27, IL17E, IL12p70, or IL1 receptor antagonist (IL1Ra) [7], [8].

IL-6, IL-8, IL-1β, and sTNFR1 were all increased in infected COVID-19 patients groups compared with Health Control subjects [9]. IL-1Ra approved in 2001 to treat rheumatoid arthritis, anakinra (IL-1Ra) has since verified to be efficacious in a broad spectrum of diseases and currently undergoing several clinical trials [10]. *In silico* pharmacology (computational therapeutics or computational pharmacology) is a rapidly growing area that globally covers the development of techniques for using software to capture, analyze, and integrate biological and medical data from many diverse sources. Specifically, *in-silico* defines the use of information in the creation of computational models or simulations that can be used to make predictions suggest hypotheses, and ultimately provide discoveries or advances in medicine and therapeutics [11]. The *in-silico* approach used in this study. Therefore, this study aimed that the IL-1Ra contained in CM Wharton's Jelly Mesenchymal Stem Cells (CM-WJMSCs) has the potential to inhibit IL-1β in COVID-19 patients through an *in-silico* approach.

Methods

The protein sequences of IL-1Ra (P18510) and IL-1β (P01584) were obtained from the database on www.uniprot.org. Both of sequences were modeled to a 3D structure using the homology modeling method on Swiss Model web-server (https://swissmodel.expasy.org/), to comparing the query sequences with the templates located on the database and resulted in homologous model formation [12]. Binding site prediction of each protein using the COFACTOR server (zhanglab.ccmb.med.umich.edu/COFACTOR) [13]. Simulation of molecular docking was performed on Cluspro's web-server (cluspro.bu.edu) and visualizing the docking results in the PyMol software to confirm IL-1β binding position with the IL-1Ra [14]. The docking results were fixed using FireDock web-server (bioinfo3d.cs.tau.ac.il/FireDock/index.html) [15], [16].

Results

This study used UniProt for sample preparation then obtained sequences sample (Figure 1) of IL-1Ra with ID P18510) and IL-1β with ID P01584. The sequences enter the 3D structure modeling stage for further analysis. After getting the FASTA format from the Uniprot website, homology modeling was carried out on the Swiss model website. Homology modeling with Swiss Model showed that IL-1RA and IL-1β have a good score for QMEAN that is IL-Ra (–1.12) and IL-1β (–1.47). Molecular visualization of protein modeling results and structural validation can be seen in (Figure 2). After attained a sample of IL-1RA and IL-1β sequences, on the Swiss-Model web-server that resulted in a modeling score for an IL-1RA protein; the value was 100% which had a similarity with the template while the value of IL-1β was 100%. The result from Ramachandran favored for IL-1RA was 95.80% and of IL-1β was 95.15%. Ramachandran plot can be seen in (Figure 3) and Ramachandran statistics can be seen in (Table 1). The quality of the protein structure is called good if the non-glycine residue in the disallow region is smaller than 15% and the smaller the percentage, the better the protein structure quality [17]. Based on the results of the IL-1RA and IL-1B analysis, it can be seen that the model is acceptable based on the non-glycine residue data that is in the disallowed area.

After getting homology modeling, it is followed by predicting binding site sites with COFACTOR software. The results of the Cscore^{LB} and BS-score can be seen in (Table 2) and the visualization of the binding site can be seen in (Figure 3). IL-1Ra protein was in the amount of 0.17 and 0.63. From the results obtained, it can be seen the IL-1Ra protein and IL-1β protein have good Cscore^{LB} and BS-score results.

a
```
>sp|P18510|IL1RA_HUMAN Interleukin-1 receptor antagonist protein OS=Homo sapiens
OX=9606 GN=IL1RN PE=1 SV=1
MEICRGLRSHLITLLLFLFHSETICRPSGRKSSKMQAFRIWDVNQKTFYLRNNQLVAGYL
QGPNVNLEEKIDVVPIEPHALFLGIHGGKMCLSCVKSGDETRLQLEAVNITDLSENRKQD
KRFAFIRSDSGPTTSFESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFYFQEDE
```

b
```
>sp|P01584|IL1B_HUMAN Interleukin-1 beta OS=Homo sapiens OX=9606 GN=IL1B PE=1
SV=2
MAEVPELASEMMAYYSGNEDDLFFEADGPKQMKCSFQDLDLCPLDGGIQLRISDHHYS
KGFRQAASVVVAMDKLRKMLVPCPQTFQENDLSTFFPFIFEEEPIFFDTWDNEAYVHDA
PVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEESNDKIPVA
LGLKE
```

Figure 1: FASTA format of protein sequences from UniProt. (a) Interleukin (IL)-1 receptor antagonist (b) IL-1β

After predicting the binding site, molecular docking is performed using Cluspro. The results of molecular docking with Cluspro can be seen in (Table 3).

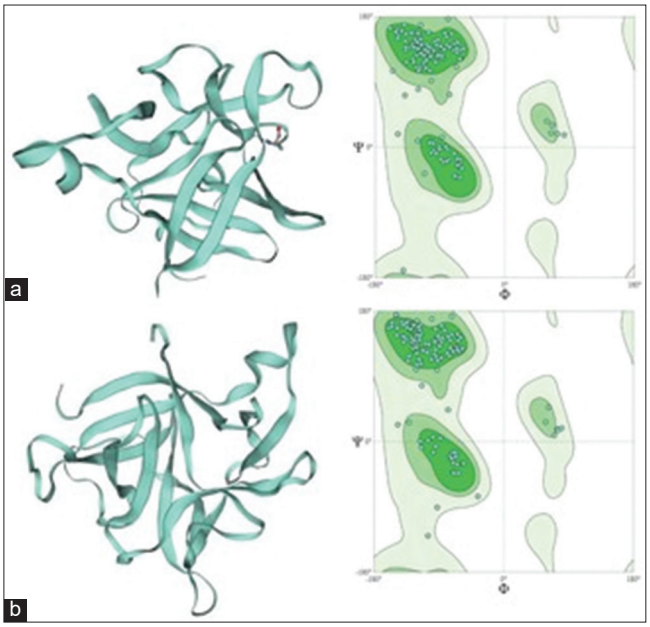

Figure 2: Molecular visualization of protein modeling results and structural validation. (a) Interleukin (IL)-1 receptor antagonist (b) IL-1β, both shown in the cartoons and chain

The lowest energy obtained was -585.1 KJ/mol. Results showed that some IL-1Ra ligand binding positions for IL-1β domains were displayed in PyMol software in the form of rigid surfaces structure with color selection (Figure 4).

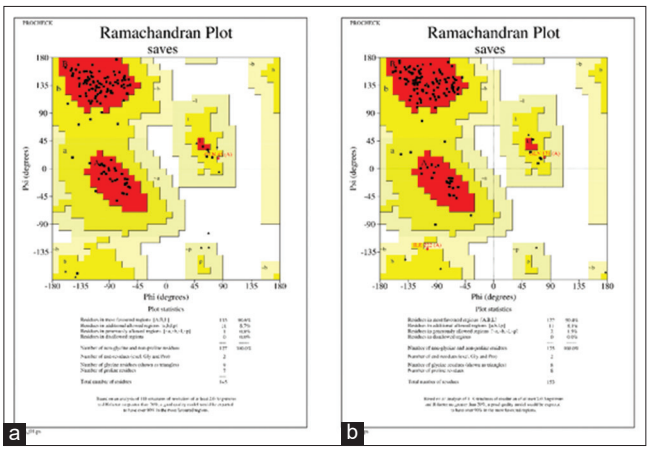

Figure 3: Ramachandran plot. (a) Interleukin (IL)-1 receptor antagonist (b) IL-1β

Energy might be required to form a stable complex between IL-1β and IL-1Ra because the molecular docking method serves to determine the shape of molecular conformation oriented to the ligand binding position in the protein-specific domain. It is predictable that IL-1Ra can inhibit or block IL-1β which causes cytokine storms in COVID-19 patients.

Table 1: Ramachandran statistics on IL-1RA and IL-1β proteins

Model	Most favored region	Additional allowed region	Generously allowed region	Disallowed region
IL-1RA	90.6%	8.7%	0.8%	0.0%
IL-1β	90.4%	8.1%	1.5%	0.0%

Next step is fixing the docking process on FireDock server. The result of fixing the docking process on FireDock server obtained about 1 ranks and obtained Van der Waals (VdW) attractive energy (VdW) in the amount of –1400.85 kcal/mol, the repulsive of 332046.49 kcal/mol, atomic contact of –399.36 kcal/mol, hydrogen bond of –249.96 kcal/mol.

Table 2: The value of CscoreLB and BS-score for IL-1RA and IL-1β proteins

IL-1RA		IL-1β	
CscoreLB	BS-score	CscoreLB	BS-score
0.52	2.01	0.51	1.98
0.06	1.18	0.06	1.33
0.04	0.80	0.03	1.07
0.04	0.85	0.03	0.92
0.04	1.11	0.03	0.83
0.03	0.90	0.03	0.81
0.03	0.90	0.03	0.86
0.03	0.91	0.02	0.85
0.03	0.96		
0.03	0.82		

Hence, it can be confirmed that the hydrogen bond interaction at rank 1 in IL-1 β- IL-1Ra complex has stability complex as it is more negative and keeps the molecule's complexes binding.

Discussion

The protein that used in this study retrieved from Uniprot. The Universal Protein Knowledgebase (UniProt) is a collection of annotations and sequences, for over 120 million proteins across all branches of life [18]. UniProt created by joining the information in PIR-PSD [19], SWISS-PROT, and TrEMBL [20].

Table 3: The lowest energy for complexes molecule IL-1Ra and IL-1β

Macromolecule	Binding position	Lowest energy (kJ/mol)
IL-1β-IL-RA	1	−541.2
	2	−585.1
	3	−500.4
	4	−534.0
	5	−537.8
	6	−507.4
	7	−521.7
	8	−533.7
	9	−495.0
	10	−539.9
	11	−481.1
	12	−504.8
	13	−496.2
	14	−535.8
	15	−505.9
	16	−479.3
	17	−485.0
	18	−504.0
	19	−494.9
	20	−467.7
	21	−480.1
	22	−462.8
	23	−460.6
	24	−484.2
	25	−483.7
	26	−461.2
	27	−461.4
	28	−464.8
	29	−455.5

Homology modeling aims to construct three-dimensional protein structure models using experimentally defined structures of associated family members as templates. SWISS-MODEL workspace is an integrated Web-based modeling expert system [21].

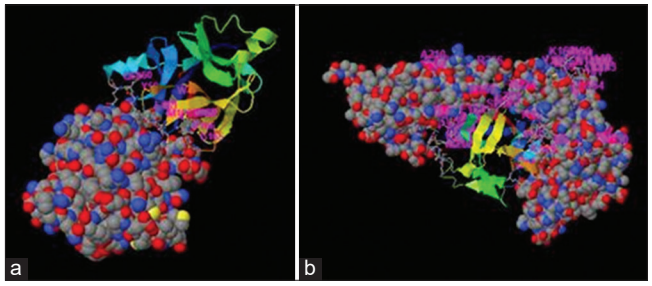

Figure 4: Structure visualization of predicted binding sites scores for a protein target (a) Interleukin (IL)-1 receptor antagonist and (b) IL-1

A score of –4.0 or lower is an indication of a low-quality model so that the results of IL-1Ra and IL-1β are categorized as good results. It is also indicated by a "thumbs up" symbol in the QMEAN result. The result of the COFACTOR is the $Cscore^{LB}$ and BS-score. $Cscore^{LB}$ is the confidence score of predicted binding site. $Cscore^{LB}$ values range in between 0 and 1, where a higher score indicates a more reliable ligand-binding site prediction. BS-score is a measure of local similarity (structure and sequence) between template binding site and predicted binding site in the query structure. Based on large scale benchmarking analysis observed that a BS-score >1 reflects a significant local match between the predicted and template binding site [13], [22].

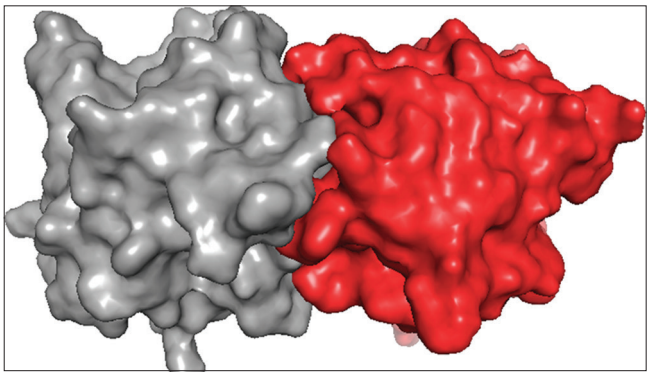

Figure 5: Structure visualization predicted of Interleukin-1 (IL) receptor antagonist (grey) as ligand inhibit IL-1β (Red) as receptor using PyMOL

The Cluspro web-server groups are the 1000 best docking models and each has different free energy. The resulted model is also grouped based on of the total value of the RMSD [23]. VdW and hydrogen bond is important for interaction between ligand and reseptor. VdW interactions divided into attractive and repulsive, both of which are sufficient to mediate enzyme binding to their substrate or antibody bonds with each specific antigen [24]. Apart from VdW interactions, hydrogen bonds also an important role in macromolecular stabilization. The type of hydrogen bonding to help stabilize the protein three-dimensional structure is nonlinear hydrogen bonds and multiple hydrogen bonds, both of them have an important role in forming large biological molecules architecture [25].

Recombinant human IL-1 receptor antagonist also called Anakinra. It is approved by the FDA for the treatment of rheumatoid arthritis and periodic cryopyrin-associated syndromes, specifically neonatal-onset multisystem inflammatory disease [26]. Anakinra will decrease IL-6 production as IL-1 is a potent inducer of IL-6, and therefore, the suggested beneficial effects of tocilizumab are likely to be seen in anakinra as well. Anakinra will not only block IL-1β but also IL-1α which is released due to epithelial and endothelial damage and thus target the inflammatory response of the tissue. Finally, the safety profile of anakinra is very good and the short half-life makes it possible to stop fast once undesired effects are seen such as neutropenia, which is not possible with tocilizumab. These arguments have led to the selection of anakinra as an immunomodulatory treatment option in several ongoing trials. A recent and larger study supports the use of anakinra in COVID-19 patients in the early phase and reports that high dose intravenous anakinra started in patients outside of the ICU was safe and resulted in clinical benefit in 72% of patients [27]. In treatment for stage III on COVID-19 patients, the use of corticosteroids may be justified in concert with the use of cytokine inhibitors such as tocilizumab (IL-6 inhibitor) or anakinra (IL-1 receptor antagonist) [28]. Furthermore, non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, celecoxib, and indomethacin commonly used by COVID-19 patients to reduce fever and relieve muscle aches, but whether NSAIDs are beneficial or harmful to COVID-19 patients is a hot topic. The use of NSAIDs so far during the COVID-19 pandemic is controversial, and caution is advised [29], [30], [31].

Conclusion

From the results of the docking of proteins between IL-1β and the CM-WJMSCs component, namely IL-1Ra, it can be predicted that IL-1Ra can inhibit IL-1β which causes cytokine storms in COVID-19 patients so that there is a potential treatment of CM-WJMSCs on the severity of COVID-19 infection.

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