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Original Article

Comparative Analysis Molecular Simulation IL6R Alpha with TCZ and HIL6: Mechanism in Inflammatory Responses

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ABSTRACT

Introduction: In cases of inflammation, there is typically a connection between IL6R and HIL6. If there is an excessive level of activity in this connection, it can lead to a cytokine storm. Tocilizumab (TCZ), also known as AntiIL-6R, is a biologic drug that is a recombinant humanized monoclonal antibody. It is specifically used to treat inflammatory and autoimmune diseases that are associated with cytokine storms. **Method**: This study utilizes in silico analysis to assess the ability of TCZ, a biosimilar, to block IL6R and compares it to the blocking effect of HIL6. Validation of the 3D structure of the IL6R was performed using a Ramachandran plot. **Results**: The IL6R alpha subunit had a validation score of 97.86%, while the IL6R beta subunit had a validation value of 95.54%. The molecular docking analysis reveals that the TCZ light chain forms a complex with IL6R, yielding a docking score of -16.4 kcal mol-1. Similarly, the TCZ heavy chain also interacts with IL6R, resulting in a docking value of -15.5 kcal mol-1. Notably, both scores are higher than the docking score of the control, which involves IL6R with HIL6, measuring -12.5 kcal mol-1. The root mean square fluctuation (RMSF) value of the IL6R protein in the presence of TCZ (Tocilizumab) is consistently below 2, with an average range of 0.04-0.09. Conclusion: The affinity between IL6R and TCZ is greater than the affinity between IL6R and HIL6.

Keywords: Binding affinity, IL6, IL6R, HIL6, Molecular docking, Molecular dynamics.

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INTRODUCTION

Inflammation is the immune system's response to pathogens, damaged cells, toxic substances, or radiation. In response to tissue injury, the body initiates a signal cascade that recruits leukocytes from blood vessels to the injured site. This activation of leukocytes leads to the production of pro-inflammatory cytokines.[1,2]

Inflammatory cytokines are responsible for recruiting leukocytes to sites of infection or injury. The classifications for these cytokines are ILS, stimulating factors (CSF), IFNs, TNFs, and TGFs.[2–5] However, excessive cytokine production during inflammation, also known as a cytokine storm, can result in tissue injury, alterations in hemodynamics, organ failure, and death. Primary inflammation is caused by the presence of microorganisms that produce the pro-inflammatory cytokine interleukin-6 (IL-6), which mediates inflammation by interacting with the IL-6 receptor (IL-6R).

IL-6 is a protein made up of 186 amino acids that promotes inflammation and is important for the immune response and the production of blood cells. The IL-6 receptor (IL6R) is a protein located on the cell membrane that facilitates the actions of IL-6 by initiating signaling pathways, such as the JAK/STAT pathway.[6–9] The disruption of IL-6 signaling has been linked to several inflammatory and autoimmune disorders, highlighting IL6R as a potential target for anti-inflammatory therapies.

It has a 3D structure, as depicted in Figure 1, which consists of four long anti-parallel α-helices (A, B, C, and D) and three interhelical loops (AB, BC, CD) of different lengths.[10] The AB and CD loops are of considerable length, however the BC loop is rather short, consisting of only three residues. The loop CD consists of a lengthy CE loop and a smaller helix E that is located outside the four-helix bundle. Helices A and B exhibit a unidirectional orientation, while helices C and D display an opposite, or counter-directional, orientation. The N- and C- termini of hIL-6 are next to each other.

IL-6 plays a significant role in the development of numerous diseases and has therefore become a crucial target for therapy.[11] The interaction between IL-6 and IL-6Rα is referred to as "classic" signaling. This interaction is responsible for the involvement of IL-6 in immunological responses to infection or tissue injury, as well as the creation of acute phase proteins by hepatocytes. On the other hand, the interaction between IL-6 and sIL-6Rα is referred to as "trans" signaling, which plays a vital role in the progression from the acute to chronic phase in several inflammatory conditions including rheumatoid arthritis, Crohn's disease, Castleman's disease, and ulcerative colitis.[12,13]

One therapy for patients with inflammation such as Rheumatoid Arthritis, autoimmune diseases, and those with inflammation and cancer is administered. Treatment techniques can be developed by either halting the generation of IL-6 or by blocking the binding of IL-6 with the IL-6R. Tocilizumab (anti IL6R) is antibodies that target the IL-6R receptor and are utilized for treating rheumatoid arthritis.[14,15] In Japan, Tocilizumab (TCZ) is also utilized for the treatment of Castleman's disease and benign B-cell tumors. In addition, tocilizumab has been recommended by the World Health Organization (WHO) for treating COVID-19 patients with bilateral lung damage and severe symptoms. Tocilizumab has more effectiveness compared to

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methotrexate immunotherapy in the treatment of juvenile idiopathic arthritis.[16]

Computational methods can greatly assist in gaining a more profound comprehension of the protein-receptor interactions that dictate the biophysics of molecular recognition.[17,18] The primary objective of our current research is to comprehend the mechanisms that regulate the binding of IL-6 to IL-6R, which initiates the signal transduction pathway and compare to binding of TCZ to IL6R. Molecular dynamics simulations are employed in this study to evaluate the conformational alterations that occur in human interleukin-6 (hIL-6) upon interaction with interleukin-6 receptor (IL-6R) and TCZ to IL6R .

The objective of this study is to conduct a comparative docking analysis of an TCZ molecule with IL6R in isolation and in conjunction with the IL6R with IL6 complex. The goals are to measure the strength of the binding, locate the areas where the binding occurs, and analyze the possible structural alterations and methods of inhibition. Through the comparison of several docking situations, we can acquire valuable knowledge regarding the mechanism by which the TCZ molecule exerts its anti-inflammatory properties. Additionally, we can determine the optimal binding mode for therapeutic applications.

OBJECTIVES

The objective of this study is to conduct a comparative docking analysis of an TCZ molecule with IL6R in isolation and in conjunction with the IL6R with IL6 complex. The goals are to measure the strength of the binding, locate the areas where the binding occurs, and analyse the possible structural alterations and methods of inhibition. Through the comparison of several docking situations, we can acquire valuable knowledge regarding the mechanism by which the TCZ molecule exerts its anti-inflammatory properties. Additionally, we can determine the optimal binding mode for therapeutic applications.

METHODS

Protein and Receptor

Receptor Structures

Retrieve high-resolution crystal structures of IL6R (PDB ID: [IP9M]), the IL6 complex (PDB ID: [1ALU]) from the Protein Data Bank and 3D TCZ with our own structure creation and validating it with Ramachandran plot.

Ensure that structures contain all pertinent chains and cofactors and are entirely complete. Utilize molecular modeling software such as PyMOL or Chimera to analyze and purify the structures, if needed.

Molecular Docking Setup

Software Selection:

Utilize a molecular docking software suite, such as H, Schrödinger Glide, or MOE, or HDOCK to conduct the docking simulations. Verify that the program is correctly set up for protein-ligand docking.2.2.[19] Preparation of Receptor and Ligand: Convert the Anti IL6R, IL6R and IL6 complex structures to the required format for the chosen docking software.

Add hydrogen atoms, assign partial charges, and define the receptor grid box around the IL6 binding site or entire receptor surface as needed.

Docking Parameters:

Define docking parameters, including the grid box dimensions, exhaustiveness, number of runs, and other relevant settings to ensure thorough exploration of potential binding modes.

Docking Simulations

TCZ with IL6R

Perform molecular docking of the TCZ molecule with IL6R alone. Run multiple docking simulations to ensure reproducibility and obtain diverse binding poses. Record binding affinities, interaction energies, and docking scores for each pose.

IL6R with IL6 Complex

Perform molecular docking of the IL6R molecule with the IL6 complex. Ensure the complex structure is intact and IL6 is bound to IL6R during the docking process. Record binding affinities, interaction energies, and docking scores for each pose.

Analysis of Docking Results

Binding Affinities:

Compare the binding affinities (e.g., binding free energy or docking scores) of the anti-IL6R molecule to IL6R and the IL6R to IL6 complex. Identify the most favorable binding modes based on the highest affinities.

Binding Sites:

Analyze the binding sites on TCZ with IL6R and the IL6R with IL6 complex to determine if the anti-IL6R molecule occupies the IL6 binding site or any allosteric sites. Visualize and compare the binding interactions using molecular visualization tools.

Conformational Changes:

Assess any conformational changes in TCZ upon binding of the IL6R molecule. Evaluate how these changes might affect IL6 binding and subsequent signaling.[20]

Inhibition Potential:

Predict the potential of the TCZ molecule to inhibit IL6R mediated signaling based on its binding mode and interactions.

Consider the implications for anti-inflammatory activity.

Validation for Computational Studies

In this research we have done molecular dynamics (MD) simulations to explore the stability of the TCZ binding poses and their impact on IL6R.[20]

RESULT AND DISCUSSION

IL6 Receptor 3D structure

The protein sequence of IL6R Alpha was obtained from the website https://www.rcsb.org/structure/1P9M. Multiple IL6R Alpha protein sequences were extracted. The aim of this study was to examine the three-dimensional structure of IL6R and its physicochemical properties. Furthermore, the objective is to determine the precise position of the binding and active site between IL6R and Tocilizumab. The purpose is to ascertain whether TCZ functions as an IL6R blocker/inhibitor and to identify the active areas of both TCZ and IL6R. The Ramachandran plot value for IL6R alpha is 97.86%, whereas for IL6R beta it is 95.54%.

Validating the 3D structure of IL6R alpha using the Ramachandran Plot to assess the precision of protein structure predictions. The Ramachandran Plot predicts the stereochemical properties of structures.[21] The Ramachandran Plot analyzes the overall geometry model with residues based on the geometry of the residues and provides information about the stereochemical quality of the predicted model. The Ramachandran Plot requires the protein model file as input

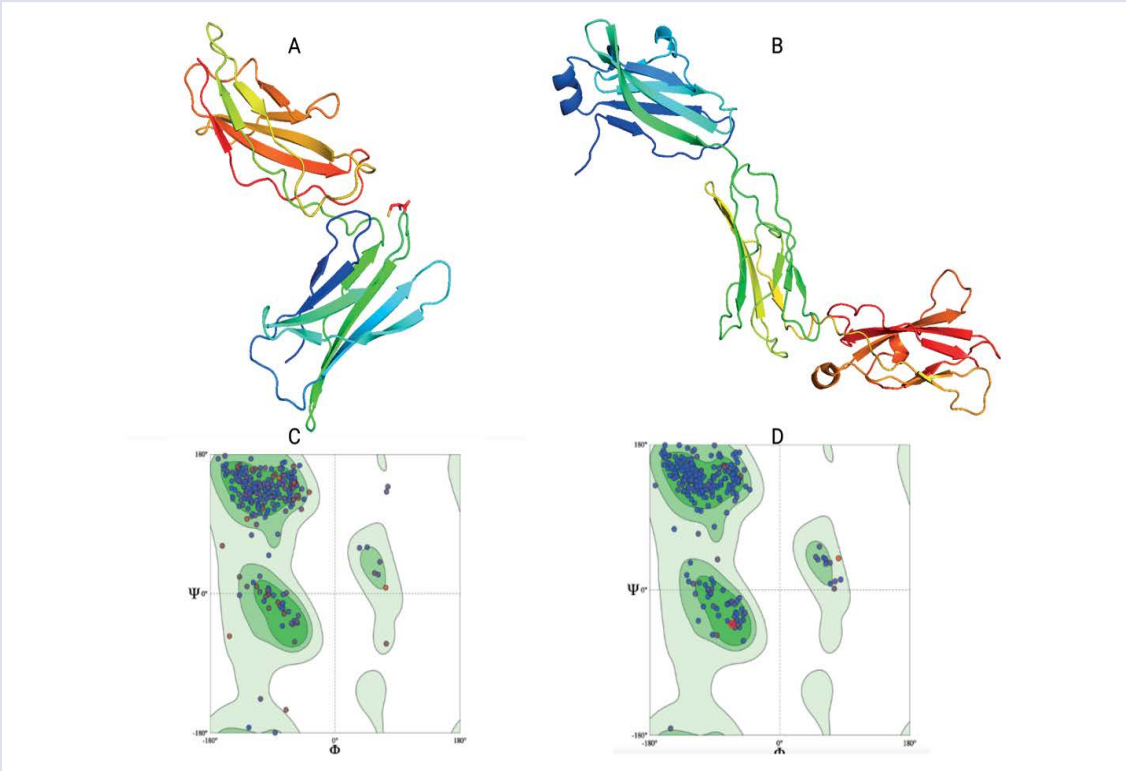

Figure 1: IL6 R alpha and IL6 R Beta. (A) IL6 R alpha (B) IL6 R Beta (C) Ramachandran Plot IL6 R Alpha (D) Ramachandran Plot IL6 R Beta.

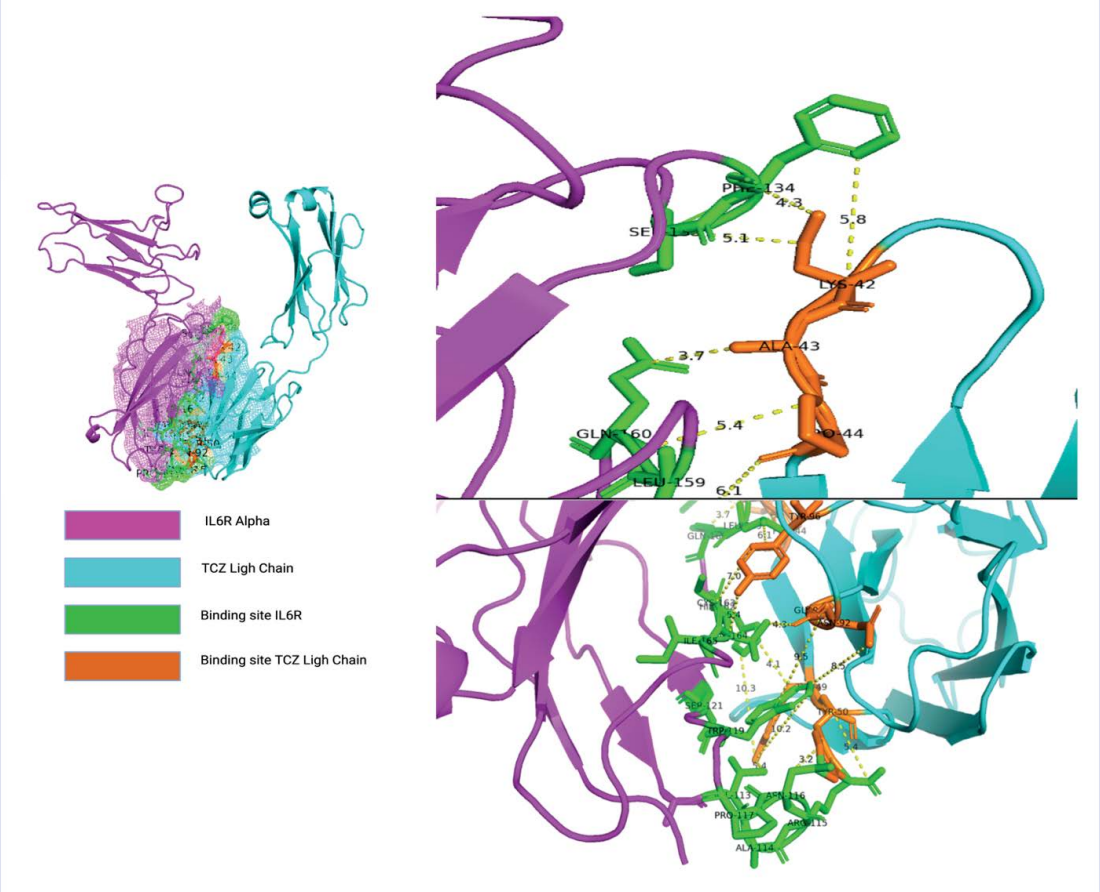

Figure 2: Molecular Docking IL6R Alpha with TCZ Ligh Chain.

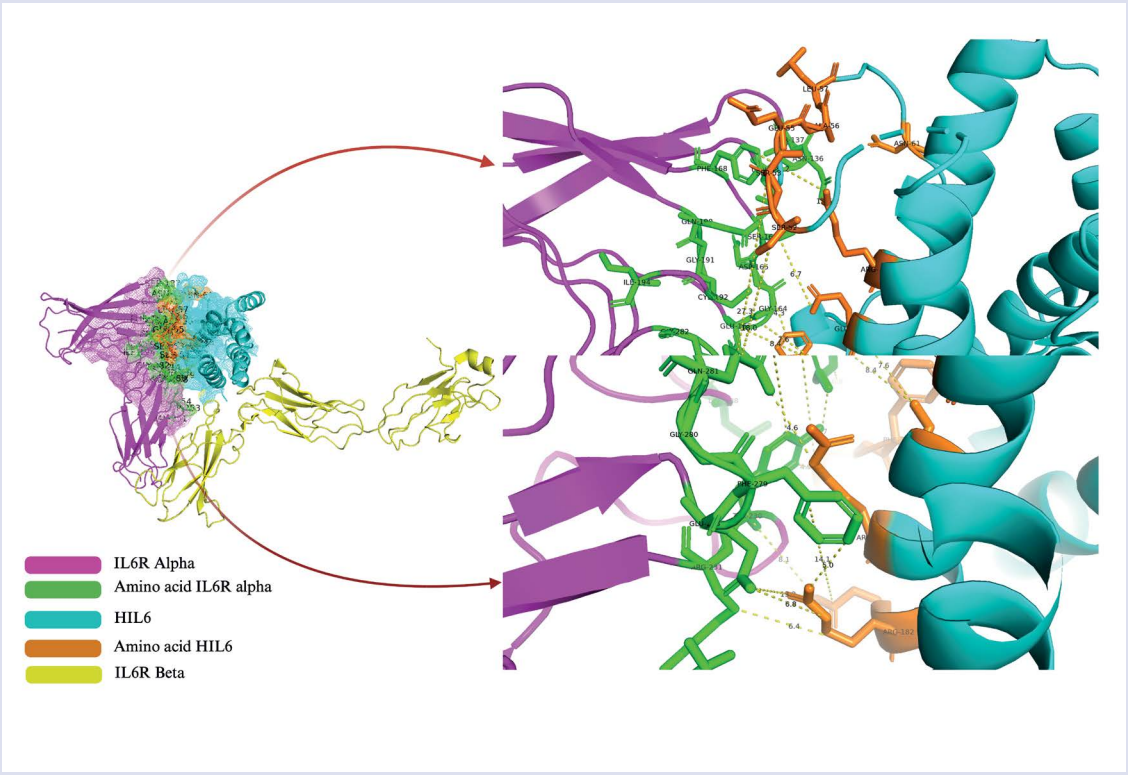

Figure 3: Molecular Docking HIL6 with IL6R Alpha.

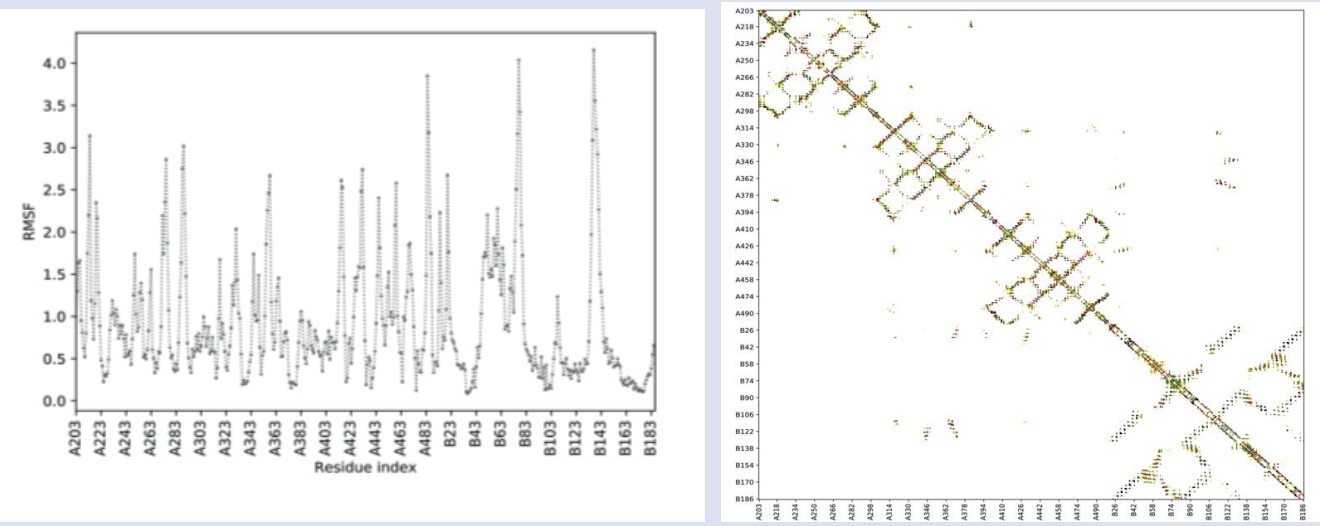

Figure 4: Molecular Dynamic Analysis.

and produces the Ramachandran plot (Fig. 1). It is anticipated that no more than 20 percent of the residue will be in the non-permitted area and that there will be no residue outside or in the non-permitted area.

The Ramachandran Plot predicts the stereochemical properties of structures. The Ramachandran Plot analyses the overall geometry model with residues based on the geometry of the residues and provides information about the stereochemical quality of the predicted model[22]. The Ramachandran Plot requires the protein model file as input and produces the Ramachandran plot. If the input structure has good resolution, then it should have a quality score of greater than 95%.

Analysis of Docking Results

TCZ with IL6R alpha

After the IL6R alpha PDB, TCZ Light chain and Heavy Chain have been acquired, proceed with molecular docking. The molecular docking of TCZ Ligh chain and IL6R alpha reveals a significant interconnection of many amino acids between the two entities, as depicted in figure 2. Figure 2 illustrates the proximity of the amino acids in the TCZ Ligh chain and IL6R alpha, with less than 5 Armstrong. Table 1 displays the specific information on the amino acids TCZ and IL6R, which form a binding interaction with each other.

Table 1. Interaction amino acid TCZ with IL6R alpha.

No	IL6R Alpha	TCZ Lighchain	Distance
1	ASN116	TYR50	3.2 Å
2	ARG115	TYR50	5.4 Å
3	VAL113	TYR49	5.4 Å
4	TRP119	TYR49; TYR50	10.2 Å; 5.3 Å
5	SER121	TYR49	4.1 Å
6	ILE165	GLY91	4.3 Å
7	VAL164	GLY91	4.3 Å
8	CYS163	TYR96	7.0 Å
9	HIS162	TYR49	4.1 Å
10	LEU159	PRO44	6.1 Å
11	GLN160	PRO44; ALA43	5.4 Å; 3.7 Å
12	SER133	LYS42	5.1 Å
13	PHE134	LYS42	4.3 Å

Table 2. Interaction amino acid HIL6 with IL6R alpha.

No	IL6R Alpha	HIL6	Distance
1	PHE168	SER53	8.2 Å
2	ASN136	ARG168	12.4 Å
3	VAL113	SER52	6.7 Å
4	TRP119	ARG168	12.6 Å
5	SER121	GLU172	6.7 Å
6	ILE165	SER53	8.3 Å
7	PHE168	SER53	8.2 Å
8	GLN135	SER57	712.6 Å
9	GLU163	SER52	18 Å

Table 3. Binding Affinity score molecular docking TCZ with IL6R and HIL6 with IL6R.

No	Description	ΔG (kcal mol-1)	Kd (M) at 25.0
1	IL6R alpha with TCZ Heavy chain	-15.5	4.3E-12
2	IL6R alpha with TCZ Ligh chain	-16.4	8.8E-13
3	IL6R alpha with HIL6	-12.5	7.1E-10

Table 1 reveals at least 13 amino acids from IL6R alpha that engage with Tocilizumab Ligh chain. The interatomic distance between these amino acids varies from 3 to 10 angstroms (Å), with an average of 4 Å. This demonstrates that the connection between TCZ and its receptor, IL6R Alpha, is highly effective at the active site region of the receptor. As a result, TCZ can prevent the interaction between IL6R Alpha and HIL6. Figure 2 provides a graphic representation of the interaction between IL6R alpha amino acids and Tocilizumab Ligh chain.

If you want to make a superior new product of TCZ, you can perform mutations in this site in the hopes of increase the binding affinity of TCZ to IL6R alpha. This active site was compared to Gupta et al.'s study, in which Glu182 IL6R alpha was identified as the active site[10]. Ala43 is the TCZ amino acid that is adjacent to Glu182 IL6R alpha beta, as determined by a molecular docking analysis.

HIL6 with IL6R alpha

After the IL6R alpha PDB, HIL6 have been acquired, proceed with molecular docking. The molecular docking of HIL6 and IL6R alpha reveals a significant interconnection of many amino acids between the two entities, as depicted in figure 3. Figure 3 illustrates the proximity of the amino acids in the HIL6 and IL6R alpha, with more than 5 Armstrong. Table 2 displays the specific information on the amino acids HIL6 and IL6R, which form a binding interaction with each other.

Table 2 reveals at least 9 amino acids from IL6R alpha that engage with HIL6. The interatomic distance between these amino acids varies from 6 to 18 angstroms (Å), with an average of 9 Å. This demonstrates that the connection between HIL6 and its receptor, IL6R Alpha, is highly effective at the active site region of the receptor. As a result, the distance between TCZ and IL6R alpha closer than HIL6 and IL6R alpha.

Subsequently, an analysis was conducted to compare the free energy scores (binding affinity) obtained from molecular docking of IL6R alpha with TCZ Light chain and heavy chain, with the free energy score (binding affinity) obtained from molecular docking of HIL6 with IL6R alpha.

Table 3 shows the results of the binding affinity study. It is evident that the docking score value of IL6 R alpha with TCZ is greater than the binding affinity of IL6R alpha with HIL6. This may be the explanation for TCZ's ability to prevent the interaction between IL6R alpha and HIL6.

Analysis of Molecular Dynamic Results

In this research we have done molecular dynamics (MD) simulations to explore the stability of the TCZ binding poses and their impact on IL6R alpha[23]. Figure 4 illustrates that the molecular dynamic value between TCZ and IL6R alpha RMSF is around ±2, just little above 2.

From the Molecular Dynamic Analysis performed to validate the good docking results between IL6R alpha and TCZ Lighchain, Figures 4 demonstrate that the RMSF value of the TCZ is 2, indicating fluctuations in amino acid residues. During the simulation process, receptor and ligand constituents are used to represent the flexibility of the residue, so it can be concluded that the lower the RMSF value, the more flexible the residue can bind .

CONCLUSION

The structural validation of IL6R alpha and IL6R beta revealed Ramachandran plot values of 97.86% and 95.54% respectively. The molecular docking analysis revealed that the binding affinity values of IL6R alpha with TCZ light chain, TCZ heavy chain, and HIL6 were -16.4, -15.5, and -12.5, respectively. Therefore, it can be inferred that the binding affinity values between IL6R alpha and TCZ were higher than the binding affinity values IL6R alpha in conjunction with HIL6. The molecular dynamic analysis results indicate that the simulation technique utilizes receptor and ligand constituents to reflect the flexibility of the residue. It may be inferred that residues with lower RMSF values have higher flexibility in binding.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

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REFERENCES

1. Tanaka T, Narazaki M, Kishimoto T. Il-6 in inflammation, Immunity, And disease. Cold Spring Harb Perspect Biol 2014;6(10); doi: 10.1101/cshperspect.a016295.
2. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The Role of Inflammatory and Anti-Inflammatory Cytokines in the Pathogenesis of Osteoarthritis. Mediators Inflamm 2014;2014; doi: 10.1155/2014/561459.

3. Fajgenbaum DC, June CH. Cytokine Storm. New England Journal of Medicine 2020;383(23):2255–2273; doi: 10.1056/nejmra2026131.
4. Liu C, Chu D, Kalantar-Zadeh K, et al. Cytokines: From Clinical Significance to Quantification. Advanced Science 2021;8(15); doi: 10.1002/advs.202004433.
5. Kang S, Kishimoto T. Interplay between Interleukin-6 Signaling and the Vascular Endothelium in Cytokine Storms. Exp Mol Med 2021;53(7):1116–1123; doi: 10.1038/s12276-021-00649-0.
6. Rossi JF, Lu ZY, Jourdan M, et al. Interleukin-6 as a Therapeutic Target. Clinical Cancer Research 2015;21(6):1248–1257; doi: 10.1158/1078-0432.CCR-14-2291.
7. Schett G. Physiological effects of modulating the interleukin-6 axis. Rheumatology (United Kingdom) 2018;57:ii43–ii50; doi: 10.1093/rheumatology/kex513.
8. Tanaka T, Kishimoto T. The Biology and Medical Implications of Interleukin-6. Cancer Immunol Res 2014;2(4):288–294; doi: 10.1158/2326-6066.CIR-14-0022.
9. Villar-Fincheira P, Sanhueza-Olivares F, Norambuena-Soto I, et al. Role of Interleukin-6 in Vascular Health and Disease. Front Mol Biosci 2021;8; doi: 10.3389/fmolb.2021.641734.
10. Gupta M, Ha K, Quarles LD, et al. Molecular Dynamics Analysis of The Binding of Human Interleukin-6 with Interleukin-6 α-Receptor Running Title: Binding of HIL-6 with IL-6Rα. n.d.
11. Su H, Lei CT, Zhang C. Interleukin-6 Signaling Pathway and Its Role in Kidney Disease: An Update. Front Immunol 2017;8(APR); doi: 10.3389/fimmu.2017.00405.
12. Nishimoto N, Terao K, Mima T, et al. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood 2008;112(10):3959–3964; doi: 10.1182/blood-2008-05-155846.
13. Kang S, Tanaka T, Kishimoto T. Therapeutic uses of anti-interleukin-6 receptor antibody. Int Immunol 2015;27(1):21–29; doi: 10.1093/intimm/dxu081.
14. Mihara M, Ohsugi Y, Kishimoto T. Tocilizumab, a humanized anti-interleukin-6 receptor antibody, for treatment of rheumatoid arthritis. Open Access Rheumatol 2011;3:19–29; doi: 10.2147/OARRR.S17118.
15. Tanaka T, Narazaki M, Kishimoto T. Anti-Interleukin-6 Receptor Antibody, Tocilizumab, for the Treatment of Autoimmune Diseases. FEBS Lett 2011;585(23):3699–3709; doi: 10.1016/j.febslet.2011.03.023.
16. Anonymous. Tocilizumab- The first interleukin-6-receptor inhibitor. n.d.
17. Bunker A, Róg T. Mechanistic Understanding From Molecular Dynamics Simulation in Pharmaceutical Research 1: Drug Delivery. Front Mol Biosci 2020;7; doi: 10.3389/fmolb.2020.604770.
18. Tiwari A, Chauhan RP, Agarwal A, et al. Molecular Modeling of Proteins: Methods, Recent Advances, and Future Prospects. In: Computer-Aided Drug Design Springer Singapore; 2020; pp. 23–47; doi: 10.1007/978-981-15-6815-2_2.
19. Mohanty M, Mohanty PS. Molecular Docking in Organic, Inorganic, and Hybrid Systems: A Tutorial Review. Monatsh Chem 2023;154(7):683–707; doi: 10.1007/s00706-023-03076-1.
20. Róg T, Girych M, Bunker A. Mechanistic understanding from molecular dynamics in pharmaceutical research 2: Lipid membrane in drug design. Pharmaceuticals 2021;14(10); doi: 10.3390/ph14101062.
21. Kumar P. Ramachandran Plot. 2018.
22. Ho BK, Brasseur R. The Ramachandran plots of glycine and pre-proline. BMC Struct Biol 2005;5; doi: 10.1186/1472-6807-5-14.
23. Yuli Astuti PD, Fadilah F, Promsai S, et al. Integrating molecular docking and molecular dynamics simulations to evaluate active compounds of Hibiscus schizopetalus for obesity. J Appl Pharm Sci 2024; doi: 10.7324/japs.2024.158550.

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