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Molecular Dynamic Stability Study of VEGF Inhibitor in Patients with Bladder Cancer

ABSTRACT

Background: Vascular endothelial growth factor (VEGF) plays a crucial role in bladder cancer progression. Brolucizumab, an anti-VEGF agent, has been studied in various diseases; however, its potential in bladder cancer remains largely unexplored. **Objective:** This study aimed to analyze the molecular docking and dynamic stability of Brolucizumab as a VEGF inhibitor in bladder cancer. **Methods:** Target protein and ligand data mining were conducted. Proteins were prepared by removing water molecules using Discovery Studio 2019. Ligand energy minimization was performed using Pyrx v.0.9.8. Protein-ligand docking was conducted, and protein-protein docking was performed using the HADDOCK server. The interactions between compounds and proteins were visualized with BioVia Discovery Studio 2019. Molecular dynamics simulations were carried out using the YASARA Dynamic program. **Results:** Brolucizumab binding induced smaller conformational changes compared to VEGFR2 binding. When VEGFR2 interacted with the VEGFA-Brolucizumab complex, significant conformational changes occurred, suggesting an inhibitory and blocking effect of Brolucizumab. Bond relaxation was observed when Brolucizumab bound to VEGFA and VEGFR, initiating conformational changes as part of its inhibitory activity. Brolucizumab demonstrated strong and competitive binding to VEGFA, with greater affinity than VEGFR2. **Conclusion:** Brolucizumab exhibits inhibitory and blocking activity against VEGFR2, suggesting its potential as a therapeutic agent in bladder cancer.

Keywords: Brolucizumab, vascular endothelial growth factor, bladder neoplasms, molecular docking simulation

1. BACKGROUND

Bladder cancer is ranked 10th worldwide, with an estimated 549,000 new cases and 200,000 deaths (1). In Indonesia, bladder cancer is included in the top 10 lists of malignancies in men, with an increasing incidence rate of 15% per year in the last decade (2).

The pathogenesis that underlies cases of malignancy including bladder cancer is angiogenesis which has implications for the development of targeted therapies that play a role in suppressing angiogenesis. Angiogenesis is the process of forming new capillaries from blood vessels that are important for cancer growth, invasion, and metastasis. Previous studies have shown that malignant tumors depend on the formation of angiogenesis for growth and metastasis (3, 4).

The main marker of angiogenesis is vascular endothelial growth factor (VEGF). In addition to inducing angiogenesis, VEGF has several additional functions that trigger an increase in tumor progression, including increasing the permeability of blood vessels in tumors, inducing serine proteases, inhibiting the process of apoptosis of endothelial cells, inhibiting the maturation of dendritic cells. VEGF can act as a factor for endothelial survival. VEGF levels from urine, blood, tissue increase significantly in bladder cancer patients (5). This study analyzed molecular docking of anti VEGF against bladder cancer. Brolucizumab as an anti-VEGF was studied in this study because studies on Brolucizumab were still limited.

2. OBJECTIVE

This study aims to analyze the molecular docking and dynamic stability of Brolucizumab, a VEGF inhibitor, in bladder cancer. Given the crucial role of VEGF in tumor angiogenesis and progression, investigating Brolucizumab's binding interactions and inhibitory po-

Model #01	File	Built with	Oligo-State	Ligands	GMQE	QMEANDisCo Global
	PDB	ProMod3 3.2.1	monomer	None	0.77	0.77 ± 0.05

Template	Seq Identity	Oligo-state	QSQE	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
6tcs.1.A	71.66	monomer	0.00	HHblits	X-ray	2.30Å	0.51	3 - 252	0.98	Omalizumab scFv

Figure 1. Brolucizumab homology model

tential may provide insights into its therapeutic applicability in bladder cancer treatment.

3. MATERIAL AND METHODS

Ligands Screening of Inhibitor Drugs

Taking information of VEGF inhibitor drugs was done using TTD database or Therapeutic Target Database (http://db.idrblab.net/ttd/) (6) and DrugBank (https://go.drugbank.com/) (7). Several anti-VEGF antibody drugs were considered for repurposing, including Ranibizumab, Aflibercept, Bevacizumab, Brolucizumab, and Faricimab. However, previous studies had already explored Ranibizumab, Aflibercept, and Bevacizumab (8). There were 2 drugs that novel, Brolucizumab and Faricimab. Therefore, we used Brolucizumab as sample.

Data Mining Target Protein and Ligand

The 3D structure of target protein was obtained from the RSCB PDB database (https://www.rcsb.org/), VGFA and VEGFR2 complex (PDB ID: 3V2A) (9). The drug's 3D structure with antibody type had to construct homology model with SWISSMODEL (https://swissmodel.expasy.org/) (10—13). This drug protein homology model then been evaluated using molprobity database (http://molprobity.biochem.duke.edu/) and ProTSAV (http://www.scfbio-iitd.res.in/software/proteomics/protsav.jsp).

Molecular Docking

Protein was prepared by removing water molecules in Discovery Studio 2019 software. Refinement was done to drug protein from the modelling using macro md_refine dari YASARA Dynamic. Protein dockings was done using Haddock server (https://wenmr.science.uu.nl/). Results from docking was obtained in the form of binding affinity or affinity energy from the interaction of compound and protein. Next, the interaction between compound and protein from docking was visualized using BioVia Discovery Studio 2019 software.

Molecular Dynamic Simulation

Molecular dynamic simulation was done using YASARA Dynamic programme developed by Biosciences GmbH. The first step was input all samples into program, then input macro to do molecular dynamic simulation which the variables were prepared such as temperature 310K and physiological pH 7,4. On the macro md_run, running time was set 10,000 ps (10ns). This simulation was done using forcefield AMBER14 (14,15). The snapshots were saved every 25 ps (16). Energy potential analysis, number of hydrogen bonds in the solute, number of hydrogen bonds between solute and solvent, RMSD, and radius of gyration was done using macro md_analyze. RMSF analysis was done using macro md_analyzeres, while Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) analysis was done using macro md_analyzebindenergy.

4. RESULTS AND DISCUSSION

Construction of Brolucizumab

Homology modelling showed that Brolucizumab had similarity with Omalizumab (Figure 1). Evaluation from MolProbity (17,18) and compilation few servers on ProTSAV (19,20) showed that homology model was good enough. However, the expected model was the model with good average evaluation result. So we carried out the refinement process using YASARA software.

Molecular Docking Analysis

VEGFA target protein is structured in a complex with VEGFR2 (VAR) (9). The sample ligand used was the antibody protein structure of Brolucizumab (VABr) which was used as VEGFR inhibitor (VABrR). The Haddock score was obtained based on the calculation in equation (1). Evdw was intermolecular van der Waals energy, Eelec was intermolecular electrostatic energy, Edesol represented desolvation energy term and Eair was water energy.

HADDOCKscore = 1.0 * Evdw + 0.2 * Eelec + 1.0 * Edesol + 0.1 * Eair.....(1)

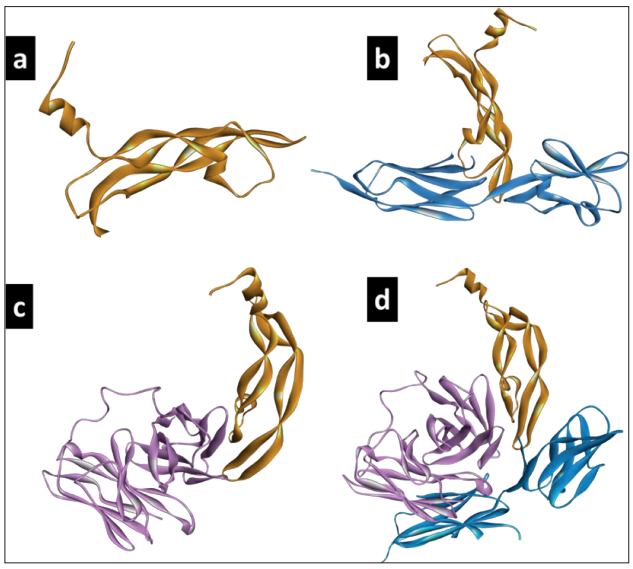

Figure 2. Visualization of docking results, a) VEGFA, b) VEGFA/ VEGFR2, c) VEGFA/ Brolucizumab and d) VEGFA/ Brolucizumab/ VEGFR2. Color description Orange (VEGFA), Blue (VEGFR2) and Purple (Brolucizumab).

	VAR		VABr		VABrR	
HADDOCK score	-86.4	-3.2	26.2	-10.1	-109.6	-11.7
Cluster size	18.0		4.0		14.0	
RMSD from the overall lowest-energy structure	7.0	0.5	17.8	0.5	1.6	1.3
Van der Waals energy	-55.1	-1.2	-46.2	-3.2	-50.1	-6.8
Electrostatic energy	-90.4	-15.5	-278.1	-9.3	-353.3	-52.6
Desolvation energy	-14.1	-1.4	-4.7	-1.5	-1.9	-3.7
Restraints violation energy	9.1	-2.2	1326.9	-76.6	130.1	-49.1
Buried Surface Area	1543.6	-28.9	1523.3	-38.5	2032	-119.7
Z-Score	-1.8		-1.9		-2.0	

Table 1. Binding affinity between VEGFA protein and sample ligands

The results of the Haddock score analysis showed that the drug Brolucizumab requires a much lower energy binding affinity for VEGFA than VEGFR2. Thus, under competitive conditions, it is estimated that Brolucizumab will more easily and stably bind to VEGFA than VEGFR2. Apart from haddock score, the comparison between VAR and VABr can also be seen based on the Z Score. The more negative the Z score, the better the complex results obtained (21). Although, VABr shows a better Haddock score and Z score than VAR. The complex between VABr and VEGFR2 (VABrR) shows a Haddock score and Z score that is more negative than VABr. However, the RMSD of the ligand, VEGFR2, increased, from 0.5 to 1.3 Å. So that the inhibition mechanism of Brolucizumab is predicted to occur by first binding to the active site of VEGFA (Figure 2c). Furthermore, VEGFR2 binds more easily to Brolucizumab which already binds to the active site than when the VEGFA active site is still exposed (Figure 2d). VEGFR2 that binds to Brolucizumab will show far more conformational changes than when it forms the VAR complex, as seen from the increased RMSD value.

Table 1 shows the interactions formed between each ligand and the VEGFA protein. The dipole-dipole bond of the hydrogan and electrostatic bond types, as well as the hydrophobic bond type is a bond formed between the target protein and the sample ligand, both VEGFR as a natural ligand and the drug Brolucizumab as a sample. Residues with bold fonts are amino acid residues from the control retained by the sample. While residues with italic fonts indicate changes in the type of bonds formed. The results of analysis of amino acid residues showed that the VEGFA/Brolucizumab (VABr) complex formed more hydrogen and electrostatic bonds than the VEGFA/VEGFR2 (VAR) complex, so it has a more negative binding affinity because the interaction occurs more stable and stronger. The results of the analysis of active site residues that are maintained by each ligand protein also support the prediction/hypothesis based on the Haddock score and Z score. The VABr complex retains 7 amino acid residues from VEGFA in the control (VAR), whereas VAAbrR only retains 3 amino acid residues from the VEGFA A domain.

RMSF analysis of amino acids residues

RMSF (Root Mean Square Fluctuation) is a score that provides information on conformational changes in more detail because it is associated with fluctuations that occur at the level of amino acid residues and nucleotide ligands. The mean RMSF of the amino acid residues of the VEGFA structure in both single samples and the VAR, VABr, and VABrR complexes showed values below 3 Å, namely 1.817 Å, 2.897 Å, 2.473 Å, and 2.59 Å respectively (Figure 3). So it is predicted that there will be no bond relaxation which can initiate the unfolding process in the conformation of the target protein. Nevertheless, at the active site amino acid residues from residue numbers 36-41 there was bond relaxation in the VArR complex which was also illustrated in the analysis of the active site amino acid residues previously, where VABrR only managed to maintain 3 residues out of 7 residues in VAR and VABr. Furthermore, the RMSF of the VEGFR2 structure showed a lower mean value when it was in the VABrR complex than in the VAR complex, which was 2.358 Å compared to 2.598 Å. The RMSF values for VEGFR2 in the VABrR complex were lower than in the VAR complex, indicating a more stable interaction with Brolucizumab-inhibited VEGFA.

These results support the Haddock score analysis, in which VEGFR2 binds more easily and stably to VEGFA which has been inhibited/blocked by Brolucizumab. In contrast to VEGFR2, the RMSF structure of Brolucizumab in the VABr complex (1.872 Å) is lower than that in the VABrR complex (2.77 Å). Brolucizumab exhibited higher RMSF values in the VABrR complex compared to the VABr complex, suggesting increased bond relaxation and conformational adaptation upon binding. Bond relaxation is predicted to occur in Brolucizumab when it binds to VEGFA as well as VEGFR so that it initiates a conformational change as an effect of its inhibitory activity.

5. CONCLUSION

Antibody based drug, Brolucizimab, was likely to have inhibition and blocking activity on VEGFR2. Brolucizumab binding more easily, quickly, and strongly to the active site of VEGFA compared to the VEGFR2.

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