In Silico Study of Vacuolin-1 as an Inhibitor of HSP27 for Precancerous Treatment of Breast Cancer

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ABSTRACT. Breast cancer has a great chance of being cured if it is diagnosed and treated properly in its early stage. The pre-cancer stage is an early stage of cancer development characterized by the overexpression of HSP27. Therefore, HSP27 can be a therapeutic target of cancer. This study aims to analyze whether vacuolin-1, a small drug compound known for its ability to inhibit metastasis, can inhibit HSP27 to prevent precancerous development in breast cancer, as well as its ADME and biosafety aspects. Protein & ligand structures were obtained from RCSB PDB and PubChem database. Preparation was performed with Discovery Studio and PyRx. Drug-likeness/ADME analysis was performed in Swiss-ADME web server. Biosafety analysis was performed in MetaTox web server. Molecular docking was performed using PyRx. The visualization of docking results was performed using Discovery Studio. The docking result between vacuolin-1 and HSP27 showed that vacuolin-1 can act as an HSP27 inhibitor by interacting with S78 residue of HSP27 and blocking its phosphorylation as well as depolymerization process. The drug-likeness characterization result of this compound showed that vacuolin-1 violates one of the four Lipinski's Rule of Five. Biosafety analysis showed that vacuolin-1 has a low toxicity level with an estimated LD50 around 13,016.65 mg/kg.

Keywords: breast cancer, HSP27, molecular docking, pre-cancer, vacuolin-1

INTRODUCTION

Cancer is the leading cause of death in the world, and the number of cancer deaths in 2020 is close to 10 million. Breast cancer is a form of cancer with high morbidity and death rates. There is a high possibility of a cure if cancer is detected and treated early on [1]. The pre-cancer stage is an early stage of cancer development characterized by overexpression of HSP27. Overexpression of HSP27 in cancer cells is associated with a poor prognosis because it protects malignant cells that cause cancer so that they cannot carry out the apoptosis process. It was also associated with drug resistance and radioresistance, resulting in poor response when patients received various treatments, including chemotherapy and radiotherapy. It is also associated with aggressive cell growth and infiltration with poor prognosis [2]. HSP27 might be a target for cancer cell treatment [3].

HSP27 itself is an sHSP (small heat shock protein) found in mammals. HSP27 in the human body is encoded by the HSPB1 gene and includes a single-copy gene with a 2.2 kb transcript, which is organized into three exons in a 205 amino acid protein pair. HSP27 is mainly located in the cytoplasm, but it can be located in the nucleus due to exposure to stress conditions. The main function of HSP27 is to regulate protein folding,

immune response, promote cancer cells, induce anti-cancer drug resistance, aging, biomarkers of various diseases, neurodegenerative diseases, cell development, and differentiation through molecular chaperone activity. HSP27 overexpression can be related to tumor occurrence and metastasis so its overexpression can lead to cancer development [4].

Vacuolin-1 is a small molecule drug that can significantly inhibit metastasis. The compound showed significant ability to inhibit the formation, migration, and invasion of various types of cancer cell colonies in vitro. Vacuolin-1 strongly inhibits lysosomal fusion of the autophagosome and lysosomal degradation of the endosome. The compound also affects focal adhesion (FA) assembly and disassembly kinetics by inhibiting the recovery and degradation of integrins. It also substantially reduced breast cancer or melanoma metastasis and/or tumor development in numerous studies or transgenic animal models [5]. This study aimed to analyze whether vacuolin-1 can inhibit HSP27 to prevent precancerous development in breast cancer as well as its drug-likeness and biosafety aspects.

RESEARCH METHODS

Receptor and ligand preparation

The structure of HSP27 (PDB ID: 6DV5) was downloaded from the PDB database

(www.rcsb.org), then the preparation was performed with Biovia Discovery Studio Visualizer v 21.1.0 to remove its ligand contaminants and water molecules. The structure of the small drug molecule vacuolin-1 (CID: 9661141) was downloaded from the PubChem database as 3D-SDF, then its energy was minimized and converted to PDB format using PyRx [6].

ADME/drug-likeness

Drug-likeness analysis, which was performed to see if vacuolin-1 meets Lipinski's Rule of Five (Ro5), was carried out in the Swiss-ADME web server (<http://www.swissadme.ch/>) [7].

Biosafety analysis

The biosafety analysis of Vacuolin-1 was performed in the MetaTox web server (<http://way2drug.com/mg>) [8].

Molecular docking & visualization

Protein-ligand specific docking was performed using Pyrx with grid center X: -164.9846, Y: 393.7237, Z:-114.4921 (HSP27-Vacuolin-1) and X:-164.87, Y: 394.572, Z:-114.6670 (HSP27-Ivermectin/control). The docking results (ligand only) are then combined with protein (macromolecules) using PyMOL software [9]. Visualization was performed with Biovia Discovery Studio Visualizer v 21.1.0 [10].

RESULTS AND DISCUSSIONS

The docking result between vacuolin-1 and Hsp27 showed that both molecules can bind to each other (Figure 1). The binding energy involved in this interaction was -3.82 kcal/mol. This interaction revealed 3 amino acids residues that bind to Hsp27. These amino acid residues are Ser78, Arg79, and Ser83. Several categories of interactions occur between vacuolin-1 and these amino acid residues including hydrophobic interaction and hydrogen bond (Table 1). These non-bond/non-covalent interactions can promote ligand binding affinity with the protein and stabilize it in certain conditions [11]. For instance, researchers have demonstrated that, hydrogen bonds may strengthen the receptor-ligand interaction formed if both electron acceptor and donor atoms have hydrogen-bonding abilities that are significantly stronger or weaker than the hydrogen and oxygen atoms present in water. On the contrary, if the hydrogen bonds present are a mixture of strong-weak H-bond pairs, interference

by bulk water can occur, causing the ligand bond affinity to decrease. [12].

Vacuolin-1 can interact and bind to the serine 78 residue in the HSP27 phosphorylation pocket. Previous studies have reported that there are three serine residues (Ser15, Ser78, and Ser82) and a threonine residue (Thr143) through which multiple kinases can phosphorylate this protein. Moreover, Ser78 and Ser82 have been reported to significantly contribute to the oligomerization of HSP27. HSP27 oligomerization is reversibly regulated by its phosphorylation and dephosphorylation events. HSP27 phosphorylation stimulates the formation of small oligomers, while its dephosphorylation stimulates the formation of large oligomers.[4].

In this study, Ivermectin, one of the anti-parasitic drugs approved by the Food and Drug Administration (FDA), known for its ability to inhibit oncogenic pathway signaling through HSP27 [13], was used as a control. Ivermectin can bind to the phosphorylation pocket of HSP27 at the same residue as vacuolin-1. This interaction inhibits the phosphorylation and depolymerization of HSP27. Molecular binding to the phosphorylation pocket of HSP27 can block HSP27-regulated survival signaling and client-oncoprotein interactions. Therefore, the binding of vacuolin-1 to this site can inhibit pre-cancerous development in breast cancer cases.

HSP27 can be found in many cell types, including adipose cells which are found in the breast. HSP27 is mainly located in the cytoplasm and can be found in the perinuclear region, endoplasmic reticulum, and nucleus [14, 15, 16]. Cytoplasmic HSP27 can be found in interphase cells. In mitotic cells, it is co-localized with mitotic spindles. During heat shock stimulation, it's translocated to the nucleus then resided in sub-nuclear structures known as SC35 speckles (nuclear splicing speckles). As previously explained, vacuolin-1 as an inhibitor of HSP27 can interact with some residues located in the phosphorylation pocket of HSP27. This direct binding, as seen in ivermectin as well, may inhibit MAPKAPK2-mediated HSP27 phosphorylation and depolymerization [17]. This mechanism occurs in the cytoplasm so vacuolin-1 doesn't need any intermediary protein or agent.

Vacuolin-1 was analyzed based on the Swiss-ADME database regarding several physicochemical properties including molecular weight (MW), LogP value, donor hydrogen bonds number, acceptor hydrogen bonds number, rotatable bonds number, and total polar surface area (TPSA) (Table 2).

Table 1. Interaction of ivermectin-HSP27 (control) and vacuolin-1-HSP27

Ligand-Protein Complex	Binding Energy (kcal/mol)	Point Interaction	Distance	Category	Types	
Ivermectin - Hsp27 (control)	0,97	C:SER78:OG - :LIG2:O	2,95299	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		D:ARG56:NE - :LIG2:O	3,1767	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		:LIG2:HO - :LIG2HO	1,78226	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		:LIG2:C – C:SER82:O	3,17478	Hydrogen Bond	Carbon Hydrogen Bond	
		:LIG2:C – C:LEU77:O	3,24069	Hydrogen Bond	Carbon Hydrogen Bond	
		:LIG2:C - :LIG2:O	3,7589	Hydrogen Bond	Carbon Hydrogen Bond	
		C:LEU77 - :LIG2	5,4245	Hydrophobic	Alkyl	
		:LIG2:C – C:LEU77	4,56104	Hydrophobic	Alkyl	
		:LIG2:C – D:ARG56	4,30842	Hydrophobic	Alkyl	
		:LIG2:C – C:LEU10	4,94325	Hydrophobic	Alkyl	
		:LIG2:C – C:LEU77	4,26871	Hydrophobic	Alkyl	
		D:TYR54 - :LIG2:C	4,9115	Hydrophobic	Pi-Alkyl	
Vacuolin-1- Hsp27	3,82	:LIG1:H – C:SER78:O	1,86251	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		:LIG1:H – C:ARG79:O	2,05026	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		:LIG1:HN – C:SER78:O	2,43165	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		:LIG1:HN – C:ARG79:O	2,66486	Hydrogen Bond	Conventional Bond	Hydrogen Bond
		C:SER83:CB - :LIG1:O	3,15669	Hydrogen Bond	Carbon Hydrogen Bond	
		C:SER78:C,O;ARG79:N - :LIG1	3,94123	Hydrophobic	Amide-Pi Stacked	
		:LIG1 – C:ARG79	3,82539	Hydrophobic	Pi-Alkyl	

The drug-likeness characterization result showed that vacuolin-1 had violated one of Lipinski's Five Rules. The violation of this rule is because the molecular weight of vacuolin-1 is higher than the rule of Ro5 (the molecular weight of vacuolin-1 is 577.42 g/mol, more than the required number of 500 g/mol), but according to Veber's provisions, vacuolin-1 is appropriate with criteria. All compounds which have the same rules as Lipinski and Veber's rules show that these compounds can theoretically be said to have ideal oral

bioavailability to be used as new drugs [17]. The drug-likeness of a compound that has the potential to become a drug can be determined using the Ro5 (Rules of five) by Lipinski and his colleagues which can show that the molecule of the drug can be distinguished based on the ability to absorb orally or not. According to Ro5, if a molecule violates two or more of these four rules, it will not have oral activity [18]. Ro5 predicts that when the donor is greater than 5H, the molecular weight is greater than 500g/mol, the calculated LogP is

Vacuolin-1 belongs to the low toxic category with a predicted LD50 of 13,016.65 mg/kg. Because vacuolin-1 is categorized as low toxic, it is almost impossible that the compound will be toxic to the human body [20]. The adverse effect of the vacuolin-1 compound is hepatotoxicity with a Pa score of 0.420. This compound cannot act as a mutagenic agent (Table 3). Prediction metabolites for vacuolin-1 come from reactions of N-Hydroxylation (1st generation to 1), N-Oxidation (1st generation to 1), N-Glucuronidation (1st generation to 2), and N-Dealkylation (1st generation to 3-14).

CONCLUSION

The small drug molecule vacuolin-1 compound is a potent inhibitor of HSP27 because it can interact and bind to the serine 78 residue in the HSP27 phosphorylation pocket. The toxicity of the vacuolin-1 is in the low toxic category with an estimated LD50 of 13,016.65 mg/kg. The detrimental effect of the vacuolin-1 compound is hepatotoxicity with a Pa score of 0.420. However, the drug-similarity characterization of vacuolin-1 shows that this compound violates one of four Lipinski's Rule of Five which is because of its higher molecular weight than the Ro5 rule.

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