Molecular Docking Analysis of α -Tomatine and Tomatidine to Inhibit Epidermal Growth Factor Receptor (EGFR) Activation in Non-Small-Cell Lung Cancer (NSCLC)

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ABSTRACT. The use of chemical drugs is the most frequent option for NSCLC therapy but most of it has side effects, such as diarrhea, skin problems, excessive body feeling, and soreness of the mouth. Therefore, NSCLC therapy using herbal medicine from plant bioactive compounds began to be developed to minimize the side effect of chemical drug consumption. Tomato (*Lycopersicon esculentum* Mill.) is one of the herbal sources which contains bioactive compounds such as α -tomatine and tomatidine. This study aimed to analyze the effectiveness of α -tomatine, tomatidine, and a combination of both to inhibit EGFR activity by molecular docking. The ligand and protein preparations were done using Discovery Studio 2016 and Hex 8.0.0 for docking. Visualization was done using Discovery Studio 2016 as well. Alpha tomatine, tomatidine, and combination of both have the potential to inhibit the interaction between EGFR and EGF as native ligands. Combination of α -tomatine and tomatidine had the best results among other complexes that have been tested with a thread of α -tomatine applied before tomatidine. Alpha tomatine and tomatidine can be considered as drugs that can control the overexpression of EGFR and decrease the activation of one of the lung cancers signaling pathways.

Keywords: alpha-tomatine, EGF, EGFR, NSCLC, tomatidine.

INTRODUCTION

Cancer is the second leading disease that causes death in the world. About 1 in 6 deaths is due to cancer [1]. Lung cancer is one type of cancer that has a high occurrence of death. Lung cancer also ranks second in the category of new cancer cases that affect both men and women [2]. One common subtype of lung cancer include non-small cell lung cancer (NSCLC) which is responsible for approximately 85-90% new cases of lung cancer [3].

Molecular markers of NSCLC are found to be overexpressed. One of which is the epidermal growth factor (EGFR). Increasing number of EGFRs on the cell surface in NSCLC patients can occur 19% to 89% from normal condition [4]. Occurrence of EGFRs overexpression is fairly high. Approximately, 62% NSCLCs is due to EGFRs overexpression [5]. Hence, choosing EGFR as a target protein will give a chance to reduce the mortality rate due to NSCLC.

Nowadays, the use of chemical medicine is a common option to undertake NSCLC therapy. Gefitinib is of that drug that specifically and efficiently targets the mutated EGFRs. However, gefitinib has side effects, such as causing diarrhea, skin problems, excessive body feeling, and soreness of the mouth [6]. Therefore, NSCLC therapy using herbal medicine from plant

bioactive compounds began to be developed to minimize the side effect of chemical medicine consumption. Tomato (*Lycopersicon esculentum* Mill.) is one of the herbal sources. Tomatoes contain bioactive compounds such as α -tomatine that has therapeutic value for lung cancer treatment and induce apoptosis [7], inhibit tumor development [8]; and tomatidine that can inhibit migration and invasion of A548 adenocarcinoma cells in human lung cancer [9]. Even if tomatidine gives effect in migration and invasion of human adenocarcinoma cells lung cancer, in this mini-project we tried to find out the effect of tomatidine before the cancer becomes malignant.

This study aimed to analyze the effectiveness of α -tomatine, tomatidine, and combination of both to inhibit EGFR activity. This project was expected to reveal the potential of tomato as herbal medicine for cancer therapy. The results can be used as additional information for NSCLC therapy using plant bioactive compounds.

METHODS

Receptor and Ligand Preparations

EGFR (ID: 1IVO) and EGF (ID: 2kv4) were downloaded from PDB database (www.rcsb.org). EGFR was prepared by Discovery Studio 2016 [10] to remove ligand contaminants and water molecules. Tomatidine (CID: 65576) ligand were

obtained from PubChem database tomatine (CID: 28523) ligand was modeled by 3D modeler [11] based on α -tomatine (ID: 28523) canonical SMILE. Ligand's energy was minimized and converted to PDB format by PyRx 0.8 [12].

Receptor-Ligand Docking and Visualization

The docking of protein-ligand was done using Hex 8.0 [13]. In this project, we established several interactions such as EGFR +EGF, EGFR + α -tomatine, EGFR +tomatidine, EGFR+ α -tomatine+ EGF, EGFR +tomatidine+ EGF, EGFR +tomatidine+ α -tomatine+ EGF, and EGFR + α -tomatine+tomatidin+ EGF. The correlation type used in this docking was Shape+ DARS. The outputs of docking were visualized by Discovery Studio 2016 [10] to analyze the interactions.

RESULTS AND DISCUSSIONS

EGF as the native ligand of EGFR interacted on several EGFR amino acid residues includes His409, Gly410, Met30, Leu38, Gly39, Gly9, Cys7, Asn40, and Val37 with total binding energy -629,3 kcal/mol (Figure 1). Nearly all of these interactions bind the EGFR Domain I in amino acids 1-133 which shows that EGF binds to the EGFR Domain participating in ligand binding [14]. Interaction between EGFR and α -tomatine established 4 hydrogen bonds and 2 hydrophobic bonds with total binding energy -507,4 kcal/mol (Table 1). Those interactions were bound with 5 amino acid residues such as Lys69, Lys270, Cys267, Lys407, and Tyr251 outside of EGFR binding site (Figure 2). Although α -tomatine was not bound in the active site of EGFR, this compound proved to inhibit the migration and invasion of lung cancer cells by inactivating FAK/PI3K/Akt and Erk signaling pathways which shown a decrease in the binding activity of NF- κ B [15]. In conclusion, α -tomatine had not the effect of inhibiting NSCLC through EGFR pathway. However, it suspected to be inhibited NSCLC through FAK/PI3K/Akt and Erk signaling pathways.

Tomatidine interacted with 6 amino acid residues with total binding energy -307.7 kcal/mol (Table 1). Tomatidine form hydrogen bonds with Arg405 and Leu38 which indicate that there is a strong bond established between the ligand and active site of EGFR located in Domains I and III (Figure 2). These results indicate tomatidine had the potential to block EGFR binding site. The EGFR-tomatidine complex interacted with EGF had total binding energy of -657.2 kcal/mol (Table 1). This complex may reduce EGFR activity by forming 2 hydrophobic bonds in Leu38 and Met87 which are the active site of EGFR (Figure

(pubchem.ncbi.nlm.nih.gov/) as 3D-SDF. The α -2). EGF which interacted with EGFR- α tomatine complex had -678.5 kcal/mol of total binding energy. A hydrophobic bond was formed at the active site of EGFR (Figure 2). Just like the EGFR-tomatidine-EGF complex, this complex may reduce EGFR activity. EGF in the compound combination complex EGFR-tomatidine- α tomatine-EGF had a total binding energy of -664.7 kcal/mol (Table 1). In this complex, EGF formed 2 amino acid bonds, conventional hydrogen bond and alkyl bond in Domains III involved Arg405 and Lys407 respectively (Figure 2). This combination also established 5 amino acid bonds in Domains II or non-binding sites. On the other hand, the complex of EGFR- α tomatine-tomatidine-EGF had a total binding energy of -669.1 kcal/mol. This interaction involved an amino acid residue Lys407 bond in binding site (Domains III) (Table 1). The thread of α -tomatine before tomatidine treatment showed the best result for compound combination to inhibit EGF binding.

Figure 1. Interaction Between EGFR with 4 domains (D-I: domain I; D-II: domain II; D-III: domain III; and D-IV: domain IV) and EGF as Native Ligand, its binding location (a) and ligand interaction (b)

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor which belongs to ErbB receptor family. EGFR also known as ErbB1 or HER1. EGFR is involved in a variety of cellular processes including cell proliferation, motility, survival, and differentiation. The aberrant activation due to upregulation of EGFR is implicated in a variety of human cancers such as non-small-cell lung cancer, metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer, and breast cancer. The receptor is activated by the binding of various ligands including epidermal growth factor (EGF). EGFR is a single-pass transmembrane protein consist of an extracellular domains, a transmembrane domains, a juxta membrane segment, a kinase domains, and a C-terminal regulatory tail. The extracellular ligand-binding domains of EGFR contains 4 distinct Domains I–IV. Both Domains I (also known as L1) and III (or L2) have a helix solenoid structure, which is

related to the leucine-rich repeat superfamily and are responsible for ligand binding through

simultaneous contact to the same bound ligand [14,16].

Table 1. Molecular interaction of EGFR amino acid residues among ligands

Interaction	Point Interaction	Distance (Å)	Type	Chemistry Bond	Energy Binding (kcal/mol)
EGFR + α -tomatine	A:LYS269:HN - :UNK0:O14	2.93	Conventional Hydrogen Bond	Hydrogen Bond	-507.4
	A:LYS270:HZ1 - :UNK0:O16	2.89	Conventional Hydrogen Bond	Hydrogen Bond	
	:UNK0:H154 - A:CYS267:O	2.52	Conventional Hydrogen Bond	Hydrogen Bond	
	:UNK0:H147 - A:CYS267:O	2.98	Carbon Hydrogen Bond	Hydrogen Bond	
	A:LYS407 - :UNK0	4.67	Alkyl	Hydrophobic	
	B:TYR251 - :UNK0:C41	5.26	Pi-Alkyl	Hydrophobic	
EGFR + tomatidine	:LIG1:H - A:ARG405:O	2.57	Conventional Hydrogen Bond	Hydrogen Bond	-307.7
	:LIG1:H - A:LEU38:O	2.42	Carbon Hydrogen Bond	Hydrogen Bond	
	:LIG1:C - A:LYS407	3.68	Alkyl	Hydrophobic	
	:LIG1:C - A:PRO272	3.98	Alkyl	Hydrophobic	
	A:PHE263 - :LIG1:C	5.30	Pi-Alkyl	Hydrophobic	
	B:TYR251 - :LIG1	5.422	Pi-Alkyl	Hydrophobic	
EGFR + α -tomatine + EGF	A:LYS269:HN - B:UNK0:O14	2.93	Conventional Hydrogen Bond	Hydrogen Bond	-678.5
	A:LYS270:HZ1 - B:UNK0:O16	2.89	Conventional Hydrogen Bond	Hydrogen Bond	
	B:UNK0:H154 - A:CYS267:O	2.52	Conventional Hydrogen Bond	Hydrogen Bond	
	B:UNK0:H147 - A:CYS267:O	2.98	Carbon Hydrogen Bond	Hydrogen Bond	
	A:LYS407 - B:UNK0	4.66	Alkyl	Hydrophobic	
	B:TYR251 - B:UNK0:C41	5.26	Pi-Alkyl	Hydrophobic	
EGFR + tomatidine + EGF	B:LIG1:H - A:THR249:O	1.67	Carbon Hydrogen Bond	Hydrogen Bond	-657.2
	B:LEU38 - B:LIG1	4.09	Alkyl	Hydrophobic	
	B:LIG1:C - B:LEU38	4.58	Alkyl	Hydrophobic	
	B:LIG1:C - B:LEU38	4.28	Alkyl	Hydrophobic	
	B:LIG1:C - B:MET87	4.52	Alkyl	Hydrophobic	
	B:LIG1:C - B:MET87	3.27	Alkyl	Hydrophobic	
	A:TYR251 - B:LIG1	4.74	Pi-Alkyl	Hydrophobic	
	A:TYR251 - B:LIG1	5.27	Pi-Alkyl	Hydrophobic	
	A:TYR251 - B:LIG1:C	4.86	Pi-Alkyl	Hydrophobic	
EGFR + α -tomatine + tomatidine + EGF	A:LYS269:HN- B:UNK0:O14	2.93	Conventional Hydrogen Bond	Hydrogen Bond	-669.1
	A:LYS270:HZ1-B:UNK0:O16	2.89	Conventional Hydrogen Bond	Hydrogen Bond	
	B:UNK0:H154 - A:CYS267:O	2.52	Conventional Hydrogen Bond	Hydrogen Bond	
	B:UNK0:H147 - A:CYS267:O	2.98	Conventional Hydrogen Bond	Hydrogen Bond	
	A:LYS407 - B:UNK0	4.66	Alkyl	Hydrophobic	
	B:TYR251 - B:UNK0:C41	5.26	Pi-Alkyl	Hydrophobic	
EGFR +	B:LIG1:H - A:ARG405:O	2.57	Conventional	Hydrogen	-664.7

Interaction	Point Interaction	Distance (Å)	Type	Chemistry Bond	Energy Binding (kcal/mol)
tomatidine			Hydrogen Bond	Bond	
+ α -tomatine	B:LIG1:H - A:LEU38:O	2.42	Carbon Hydrogen Bond	Hydrogen Bond	
EGF	B:LIG1:C - A:LYS407	3.68	Alkyl	Hydrophobic	
	B:LIG1:C - A:PRO272	3.98	Alkyl	Hydrophobic	
	A:PHE263 - B:LIG1:C	5.30	Pi-Alkyl	Hydrophobic	
	A:PHE263 - B:LIG1:C	4.60	Pi-Alkyl	Hydrophobic	
	B:TYR251 - B:LIG1	5.42	Pi-Alkyl	Hydrophobic	

Figure 2. Interaction of EGFR with 4 domains (D-I: domain I; D-II: domain II; D-III: domain III; and D-IV: domain IV) and ligands. Left section shows the binding location among EGF (yellow), α -tomatine (red), and tomatidine (green) to EGFR (blue). Middle section shows ligands interaction with amino acid residues of EGFR. Right section shows 2D diagram of the interaction

Domains II (or CR1) and IV (or CR2) are both cysteine-rich regions with disulfide bonds similar to those seen in laminin and the tumor necrosis factor receptor. Its intracellular Domains residues are known to be phosphorylated. These phosphotyrosine residues bind soluble or membrane-anchored effector proteins that are recruited upon receptor activation. EGFR activates several downstream signaling cascades, which include pathways mediated by Ras/Raf/MAP kinase, phosphoinositide-3-kinase (PI3K)/Akt, and phospholipase C γ . Upon ligand binding, the C-terminal tail becomes tyrosine-phosphorylated and mediates the interaction between the receptor and downstream effectors [14, 16].

In this study showed that tomatidine and combination of both tomatidine and α -tomatine can bind in the active site of EGFR which means they can inhibit EGFR to bind with its ligands. This interaction may downregulate the activation of EGFR in cancer especially NSCLC. Alpha tomatine did not bind in the active site independently but this compound has the potential to inhibit EGFR when combined with tomatidine. Previous studies showed that tomatidine which

CONCLUSION

The α -tomatine and tomatidine have potential as inhibitors of the interaction between EGFR and EGF as native ligands. These two compounds were proven to bind with EGFR in their ligand binding site. Combination of α -tomatine and tomatidine had shown the best results among other

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