In Silico Analysis of Formononetin Compound as a Breast Anti Cancer

Análisis en silico del compuesto de formononetina como un anticáncer de mama

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Abstract

For long the relationship between estrogen and cancer has been proved. Formononetin is a phytoestrogen which has been reported to have anticancer effects. Phytoestrogens have the same structure and properties as human estrogen and can interact with ER-α without inducing any cell proliferation. While anticancer properties of Formononetin in prevention of other cancers like prostate and carcinoma colon have been studied, its effects on breast cancer remain unknown. The aim of the present study is to analyse the formononetin compound as a competitor of estrogen in terms of the bond quality with ER-α. The method used in this study was in silico or computation by molecular docking method using PvRx software. The data was also analysed using Discovery Studio software. The results showed that the formononetin compounds have a value of free energy equal to -7.3 kcal/mol, lower than 17-β-Estradiol (natural estrogen) that is -6.4 kcal/mol, but higher than 3 alkylnaphthalene (estrogen synthetic) that is -11.2 kcal/mol. Based on the results, formononetin has a great potential as an estrogen competitor because it has a value of free energy that is lower than the natural estrogen.

Keywords: Breast cancer; estrogen; formononetin; in silico modeling

Resumen

Durante mucho tiempo se ha comprobado la relación entre estrógeno y cáncer. La formononetina es un fitoestrógeno que se ha informado que tiene efectos anticancerígenos. Los fitoestrógenos tienen la misma estructura y propiedades que el estrógeno humano y pueden interactuar con ER-α sin inducir ninguna proliferación celular. Si bien se han estudiado las propiedades anticancerígenas de Formononetin en la prevención de otros cánceres como la próstata y el carcinoma de colon, sus efectos sobre el cáncer de mama siguen siendo desconocidos. El objetivo del presente estudio es analizar el compuesto de formononetina como un competidor del estrógeno en términos de la calidad del enlace con ER-α. El método utilizado en este estudio fue in silico o computación mediante el método de acoplamiento molecular utilizando el software PvRx. Los datos también se analizaron utilizando el software Discovery Studio. Los resultados mostraron que los compuestos de formononetina tienen un valor de energía libre igual a -7.3 kcal/mol, menor que 17-β-estradiol (estrógeno natural) que es -6.4 kcal/mol, pero mayor que 3 alquilnaftaleno (sintético de estrógeno) Eso es -11.2 kcal/mol. Basado en los resultados, formononetin tiene un gran potencial como competidor de estrógeno porque tiene un valor de energía libre que es más bajo que el estrógeno natural.

Palabras clave: cáncer de mama; estrógeno; formononetina; modelado in silico

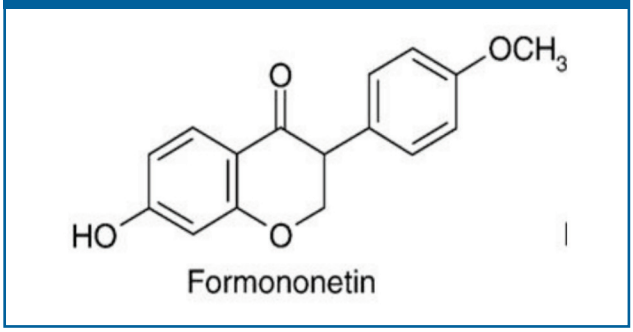
Introduction

Cancer is a disease characterized by abnormal cell growth in body tissues with mutations and changes in the biochemical structure[1]. Breast cancer is a type of cancer that is often encountered in women, world-wide, representing 16% of all types of cancer suffered by women. 519,000 deaths were reported due to breast cancer[2]. Breast cancer begins with abnormal cell division of breast tissue that is capable of invading and distributing in other organs[3].

Breast cancer can be caused by other factors such as estrogen and estrogen receptors. Estrogen is a hormone that is very closely related to female reproduction. However, high levels of endogenous estrogen as well as hormonal therapy with estrogen for menopause are linked to higher risk of breast cancer[4]. Estrogen is able to stimulate the proliferation of epithelial cells in the breast gland by increasing estrogen receptor which mediates transcription of genes. Increased estrogen in the female body will result in hyperproliferation, and thus can potentially cause breast cancer[5].

Phytoestrogens are largely distributed in plants and have similar structures and functions with human estrogen; therefore, they have the capability to bind to estrogen receptors[6]. There are three main classes of phytoestrogens namely isoflavones (which mostly can be found in soybeans and soy products); lignans (which widely can be found in seeds, whole grains, berries, fruit, vegetables, and nuts); and coumestans (which are distributed in broccoli and sprouts). Phytoestrogens bind weakly to estrogen receptors, yet they do not cause cell proliferation reactions. Although the role of phytoestrogens in human body is ambiguous, they are reported[7] to be protective against breast cancer owing to their structure (Potential, see conclusion). Therefore, phytoestrogens are one of the potential compounds that act as estrogen competitors in the body to reduce cell proliferation. One of the potential phytoestrogens is formononetin. Formononetin is an isoflavone and can be found in several types of plants such as soybeans, katuk leaves, and red clover. Figure 1 shows is the structure of formononetin:

Figure 1. Structure of formononetin[8]



Formononetin as an effective anticancer prevents prostate cancer and carcinoma colon[9], but its role in breast cancer is unknown.

The most common treatment methods for breast cancer are surgery, chemotherapy, hormonal therapy, and immunologic therapy. However, they have many disadvantages. For instance, although chemotherapy kills cancer cells, it also negatively affects normal cells with rapid proliferation rates, such as hair follicles, bone marrow and digestive tract cells, resulting in typical chemotherapy side effects such as hair and skin loss or skin dry up[1]. Therefore, researchers are looking for more effective treatments with minimum side effects. In line with this, one approach is exploring anticancer compounds like phytoestrogens from natural sources. This study aims to determine bonds between formononetin and estrogen receptor alpha (ER-α) and to examine the potential of formononetin as an estrogen competitor in the human body using the in silico method.

In silico is a term used to refer to computational research work. This method is often used to find new drugs by predicting the interaction between the candidate drug compounds (ligand) and the target receptor. There are many computational methods that can be used and one of them is molecular docking[10]. Molecular docking helps to predict the natural process which occurs within seconds in a cell[11]. It is a computational procedure used to predict conformation of proteins or molecules of nucleic acids (DNA or RNA), and ligands. The process involves placing molecules in appropriate configurations to interact with a receptor. The molecular docking algorithms execute quantitative predictions of binding energetics, and provide rankings of docked compounds based on the binding affinity of ligand-receptor complexes[11].

Methods

Research Design

This study was an experimental research using the in silico approach. The instrument used in this research were the PyRx and BIOVIA Discovery Studio 2017 R2 softwares. The materials used in the experiment were the three-dimensional structure of formononetin in mol2 form, estrogen receptors alpha (natural estrogen) and three-dimensional 3-alkyl naphthalene (synthetic estrogen). Formononetin was obtained from zinc.docking.org under the code ZINC18847036, the estrogen receptors alpha were downloaded from http: //www.rcsb.org/pdb/home/home.do using 3DT3 code in the .pdb format and 3-alkyl naphthalene can be downloaded at pdb.org using 3DT3 code.

The in silico method employed in this study includes molecular docking of proteins and ligands to predict ligand binding model in the dominant area known as protein in a three-dimensional structure[12]. This research is carried out computationally, consisting of the following steps:

Preparation of test compound

In this study three compounds consisting of three ligands and one receptor were tested. The three ligands consisted of one test ligand namely formononetin and two control ligands namely 17-β-estradiol (natural estrogen) and 3-alkyl naphthalene (synthetic estrogen). The receptor used are estrogen receptors alpha (ER-α). All ligands should be saved in the mol2 format that is suitable for PyRx software. The ER α should be separated by their innate ligand, so that they will not overlap with the test ligands when molecular docking is carried out.

Molecular Docking Process

Molecular docking is a process of uniting or combining of test compounds with their receptors. The combination process was carried out using the PvRx software. The outcome from this process which was the information on the conformation and binding affinity value (free energy) of the ligands would be further analysed using BIOVIA Discovery Studio 2017 R2 software[13]. Conformation is a three-dimensional image of interactions between test ligands and ER-α which results from molecular docking processes. Based on the result of conformation, the number and type of bond formed between the test ligand and ER-α will also be identified. The binding affinity value i.e. free energy values, show the bond calculation formed between the test ligand and the receptor.

Analysis Molecular Docking Results

The conformation and binding value of free energy obtained from molecular docking process were then visualized using BIOVIA Discovery Studio 2017 R2 software. The value of binding affinity is always negative because it describes the initiation between ligands and receptors. Low value of binding affinity would indicate a good bond quality. In this process also, the location of the tested ligands in the alpha ER-α was identified. In addition, the number and type of bond formed between the test ligand and ER-α will also be scrutinised.

Results and Discussion

Estimation of the binding mode of a ligand inside the binding site of a protein of known structure is very important in rational drug design. Molecular docking simulations on the crystal structure of estrogen receptor were employed in order to study in silico the ability of the studied test ligands to bind to the said receptor.

Figure 2. Comparison of conformations of third ligands (formononetin, 3-alkyl naphthalene, and 17-β-estradiol).

The findings of the analysis have shown that all the three tested ligands indicate positions that are similar to the ER-α (estrogen receptors alpha). Based on Figure 2 formononetin (blue), 3-alkyl naphthalene (red) and 17-β-estradiol (green) are on the active site of the ER-α. This indicates that all three test ligands are in the correct position on the receptor.

Table 1 below illustrates the number of interacted bonds and the types of bonds between the tested ligands and ER-α.

Table 1. Comparison of number of interaction bonds

No	Name of Ligand	Bond		
		Hydrogen Bond	Hydrophobic Bonds	Other Bond
1	Formononetin	-	8	-
2	3-alkyl naphthalene	3	14	-
3	17-β-estradiol	-	5	1

Based on Table 1, the interaction formed between formononetin and ER-α is a hydrophobic bond and consists of 8 bonds, while the interaction between 3-alkyl naphthalene and ER-α forms 3 hydrogen bonds and 14 hydrophobic bonds. The interaction between 17-β-estradiol and ER-α forms 5 hydrophobic bonds and 1 other bond.

Figures 3, 4, and 5 below provide a further information regarding the bonds that have been formed. ER-α is a protein that composed by amino acids. Figures 3, 4, and 5 show the amino acids from ER-α which are bound to each test ligand. The number of amino acids bound to the test ligand illustrates the number of bonds formed between the test ligand and ER-α.

Figure 3. Visualization of formononetin interactions (green) with estrogen receptor alpha (red).

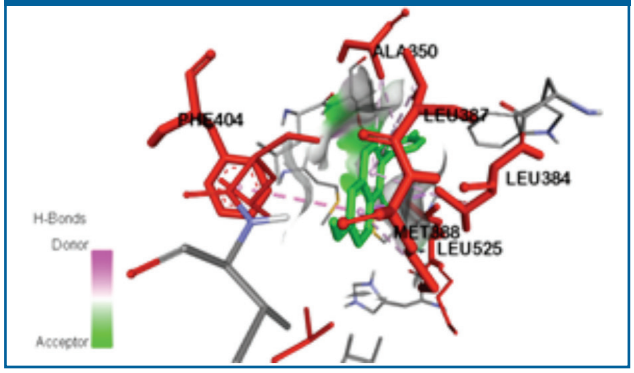


Figure 3 shows the bond between formononetin compounds and the residue (amino acid of receptor). There are 6 residues in the ER-α compound (in red) consisting of LEU384 (Leucine 384), LEU525 (Leusin 525), PHE404 (Phenylalanine 404), ALA350 (Alanine 350), LEU387 (Leusin 387) and MET388 (Methionine 388). The numbers that follow the name of amino acid is the sequential numbers in the ER-α structure.

Figure 4 shows the bond between 3-alkyl naphthalene compounds and the residues (amino acid of receptor). There are 12 residues that are bound to ER-α including GLU353 (Glutamic Acid 353), GLY521 (Glycine), PHE404 (Phenylalanine), LEU346 (Leucine), MET388 (Methionine 388), LEU391 (Leucine 391), LEU428 (Leucine 428), ILE424 (Isoleucine 424), LEU525 (Leusin 525), ALA350 (Alanin 350), LEU349 (Leusin 349), LEU387 (Leusin 387).

Figure 4. Visualization of 3-alkyl naphthalene interactions (blue) with estrogen receptor alpha (red).

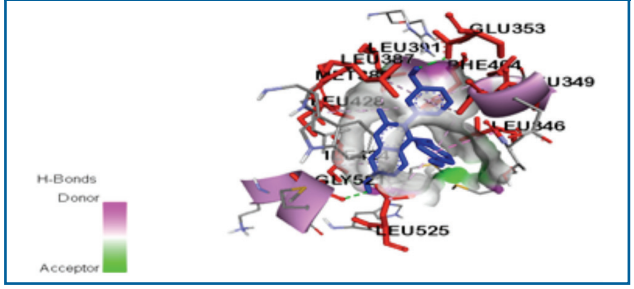


Figure 5. Visualization of 17-β-estradiol interactions (red) with estrogen receptor alpha (blue).

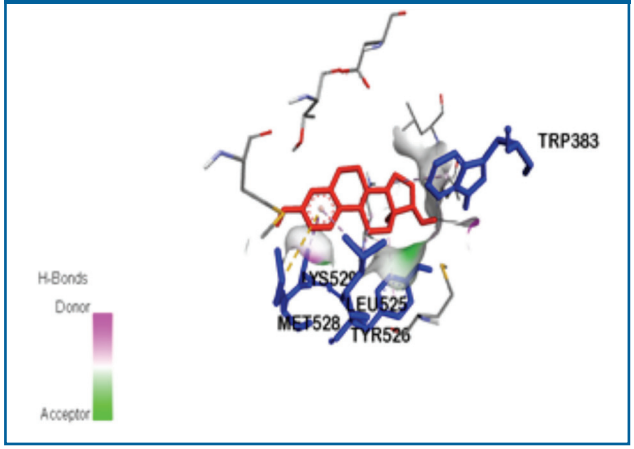


Figure 5 shows the bond between 17-β-estradiol compounds and the residues (amino acid of receptor). There are 5 residues in this compound is 5 that are bound to ER-α including TRP383 (Tryptophan 383), LEU525 (Leucine 525), TYR526 (Tyrosine 526), LYS529 (Lysine 529), and MET528 (Methionine 528).

Calculation of Affinity Binding: The affinity binding values indicate the quality of affinity (bond strength) between the tested ligands and ER-α. Table 2 below illustrate the values obtained from the analysis.

Table 2. The affinity binding value (kcal / mol) of formononetin, 3-alkyl naphthalene and 17-β-estradiol with estrogen receptor alpha

Table 2. The affinity binding value (kcal / mol) of formononetin, 3-alkyl naphthalene and 17-β-estradiol with estrogen receptor alpha

No	Name of Ligand	Binding Affinity (kcal/mol)
1	3-alkyl naphthalene	-11.2
2	Formononetin	-7.1
3	17-β-estradiol	-6.4

The bond strength between the ligand and its receptors is predicted by the value of binding affinity obtained from the docking results. The value of binding affinity is the power of interaction between two molecules or more. The greater the value of binding affinity is, the lower the bond between the receptor and the ligand will be[14].

Based on Table 2, the formononetin compound shows lower free energy (-7.1 kcal/mol) compared to 17-β-estradiol (-6.4), but higher when compared to 3-alkyl naphthalene (-11.2). This seems to suggest that formononetin has the potential to become the competitor of estrogen in the body, because the estrogen in the body is 17-β-estradiol. According to the literature[14], the bonding of a compound can be considered to be good or strong if the value of binding affinity is lower because if the binding affinity is low then the bond formed is more stable, when compared with the high binding affinity value. Therefore, the higher value of binding affinity will result in less stable interaction between the bonds.

Conclusions

The compound of formononetin is a phytoestrogen compound capable of binding to estrogen receptor alpha (ER-α), with free energy value of -7.1 kcal/mol, lower than the control compound of -6.4 kcal / mol. However, formononetin is no better than that of other 3- alkyl naphthalene control compounds with free energy values of -11.2 kcal / mol. Based on the results, the compounds of formononetin has the potential to be an active ligand with the estrogen alpha receptor (ER-α). For

future studies, more tests on formononetin compounds using the in vivo and in vitro methods can be conducted to test the stability of interaction between formononetin and the estrogen alpha receptor (ER-α) through molecular dynamic tests.

Based on the molecular docking results, the formononetin compounds have a better bonding quality to alpha estrogen receptors than the natural estrogens (17-β estradiol), but no better when compared with naphthalene. This indicates that formononetin has the potential as a natural estrogen competitor and therefore, it could be a candidate to be used as breast anti cancer drug.

Rational drug design of phytoestrogen derivatives unlocks new outlooks to selectively utilize the health beneficial properties of this natural compound for the treatment of breast cancer. The results of present study can be used as a reference for future studies on alternative treatments for breast cancer. Our findings have shown that formomonetin has the capability of prevention and/or treatment of cancer. The use of formononetin as an alternative treatment in breast cancer may reduce the high rate of mortality in breast cancer patients.

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