Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 1, 2015

Original Article

WALNUT PEDUNCULAGIN A PROBABLE SERM FOR BREAST CANCER TREATMENT

SHYAM PERUGU*, RADHIKAVEMULA, VISHNU VARDHANA RAO M*

***Scientist, Biomedical Informatics Centre, National Institute of Nutrition (ICMR), Jamai-Osmania PO, Hyderabad-500 007, Telangana, India.**

Email: shyambioinf@gmail.com

Received: 01 Oct 2014 Revised and Accepted: 30 Oct 2014

ABSTRACT

Walnuts constituents have been actively used in nutrition in slowing cancer growth by its anti-proliferative and anti-angiogenic mechanisms. Pedunculagin, a natural chemical constituent of walnut were performed to check for its binding with Estrogen Receptor (ER) receptor using Insilco approach. Most of current approach towards breast cancer therapies is aimed at blocking ER signaling pathway using various estrogen antagonists like synthetic estrogen receptor modulators (SERMs) which down regulates the signaling cascade leading to tumor activity. This study is aimed to find the binding interactions between Pedunculagin and ER. Pedunculagin structure was extracted from CHEMSPIDER database and Estrogen Receptor (ER) 1QKN was selected, further ligand and receptor structures were minimized with steepest descent and conjugate gradient with minimized energy levels were found to be -14211.04235 Kcal/mol for ligand molecule and -12518.66882 Kcal/mol for ER. Six interacting binding sites were identified between ligand and ER and docking studies showed second active binding site of receptor showed strong affinity and interacting groups. Pedunculagin may act as SERM in modulating the ER signaling activity and may be a probable therapeutic molecule for treating breast cancer.

Keywords: Breast cancer, Walnut, Pedunculagin, Docking and anti–angiogenesis.

INTRODUCTION

Chemotherapeutic way of treating breast cancer has been a major challenge with the drug efficacy and other side effects. Recent years has seen use of selective estrogen receptor

modulators (SERMs). Many breast cancers are sensitive to the hormone estrogen. This means that estrogen causes the breast cancer tumour to grow [1]. Such cancers have estrogen receptors on the surface of their cells. They are called estrogen receptor-positive cancer or ER-positive cancer. Breast cancer may also occur due to mutations in tumour suppressor genes and in genes which play a vital role in cell cycle control.

Breast cancer growth is majorly of two types like estrogen dependent breast cancer and HER2 dependent breast cancer. SERMs have either selective agonist or antagonist effects, depending on the target tissue. The molecular basis of SERM action and the tissue-selective agonist–antagonist effects is very useful in studying about the treatment strategies for curing breast cancer among women. Tamoxifene and Raloxifene are the mainly used SERMs in treating cancers in recent years.

However research has been active in finding the best SERM having minimum side effects and showing a uniform antagonist action in broad range of tissues of the body. Plant derived flavonoids and other poly unsaturated cyclic compounds have reported to show estrogen receptor binding activity. Studies on diet containingwalnut have shown to decrease the breast cancer tumour growth in animal models. However the molecular basis of chemical constituent of walnuts showing anti – cancerous activity in breast tissue is unknown.

Statistics

According to the National Cancer Institute, USA. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. 18.2% of all cancer deaths worldwide, including both males and females, are from breast cancer [2]. There are 2, 32,340 female breast cancers patients and 2,240 male breast cancers patients are reported in the USA every year, as well as about 39,620 deaths caused by the disease. The overall estimate of 1,665,540 new cases is the equivalent of more than 4,500 new cancer diagnoses each day. About 62,570 cases of breast carcinoma in situ and 63,770 cases of melanoma in situ are expected to be newly diagnosed in 2014.

Chemical product description

A walnut is an edible seed of the genus Juglans (Juglandaceae Family), especially the Persian or English walnut, Juglansregia [3]. Walnuts contain high density source of nutrients, particularly proteins and essential fatty acids. Raw walnuts contain glyceryltriacylates of the n-3 fatty acid alpha-linolenic acid (ALA), which is not as effective in humans as long-chain n-3 fatty acids, and (mostly insoluble) antioxidants [4]. The antioxidant properties of walnuts help lower risk of chronic oxidative stress, and the anti-inflammatory properties help lower risk of chronic inflammation, and it is precisely that when these two types of risks combined, pose the greatest threat for cancer development[5]. Prostate cancer and breast cancer are the best-studied types of cancer with respect to walnut intake, and their risk has been found to be reduced by fairly large amounts of walnut consumption [6].For prostate cancer, the evidence is somewhat stronger, and more studies have involved human subjects. For breast cancer, most of the evidence has been based on studies of rats and mice. Walnuts are also dense with antioxidants and essential amino acids. A 2002 study conducted in Norway showed that walnuts rank second only to rose hips in their antioxidant content. Ellagic acid, an antioxidant, supports the immune system and has anticancer properties. L-arginine, an essential amino acid, is converted to nitric oxide in the body, helping to keep blood vessels smooth and relaxed. Walnuts contain the phytosterols and Pedunculagin etc, which may probably bind to estrogen receptors and therefore may prevent or slow the growth of breast cancer tumours fueled by estrogen [7].

In animal studies, walnuts cut the risk of breast cancer [8]; when it did develop, walnuts helped curb tumourgrowth [9]. A phytoestrogen is a naturally-occurring plant nutrient that exerts an *estrogen-like* action on the body [10]. Scientists have discovered hundreds of phytoestrogens including soybeans, whole grains, seeds (especially flax), nuts (especially walnuts) and many herbs. Walnut diet significantly improved endothelium-dependent vasodilation [11]. There is evidence showing that vascular endothelial function is markedly influenced by estrogen and is improved by hormone replacement therapy in postmenopausal women.

MATERIALS AND METHODS

From the walnut, Pedunculagin ligand is identified and was selected for docking studies. 3D structures of ligand were retrieved from

PUBCHEM and CHEMSPIDER, this structure was drawn by using "CHEMSKETCH". The ligand was prepared and minimized by using Accelrys Discovery Studio v2.1 [12]. The crystal structure of estrogen receptor was downloaded from the PDB data bank (PDB-ID: 1QKN). Structure was crystallized by using X-ray crystallography and it has 2.25A° resolution.

The side chains of receptor were then minimized using Accelrys discovery studio. The initial energy was -14211.04235 Kcal/mole 1100 steps of conjugate gradient and end energy was -14211.04235 Kcal/mole 1200 steps of conjugate gradient. Active Binding Site (ABS) of the subunits were predicted using ADS and the protein-ligand docking [13] was performed on ADS using Ligand Fit module with empirical scoring functions and results were obtained with respect to highest dock score, which is >20. The 3D structure was shown in figure.1 by using SSViwer [14].

RESULTS

The ligand structure was minimized with steepest descent and followed by conjugate gradient and found to be stabilized at 1200 steps with -14211.04235 kcal/mol. We found the second active binding sites of estrogen receptor and found to be good interactions with ligandpedunculagin and docking score -108.0204 kcal/mol. The table: 1 represents the energy minimization of receptor and about their potential energies.

Table 1: Energy Minimization of receptor structure

Force field	Initial Potential Energy (kcal/mol)	Potential Energy(kcal/mol)	Van der Waals Energy(kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/mol x Angstrom)	Final RMS Gradient(kcal/mol x Angstrom)
CHARMm	2124227.4056	-14211.04235	-1549.64292	-14477.41456	299797.6774	0.28563

The energy minimized ligand structures were uploaded to Accelrys discovery studio along with the estrogen receptor and analysed the interactions as described in the materials and methods.

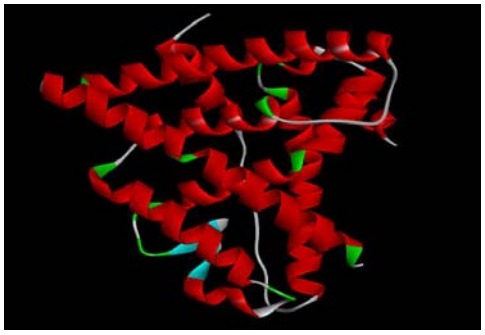
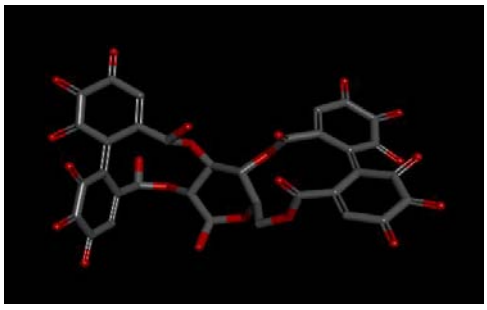


Fig. 1: energy minimized receptor structure



Receptor structure is downloaded from pdb databank (1QKN. pdb). The 3D structure was shown in fig. 1 Helices were represented in red colour, loops are represented in white colour and beta sheets are shown in cyan colour.
Fig. 2: 3D Structure of Ligand Pedunculagin

Ligand pedunculagin is extracted from CHEMSPIDER database. It is shown in sticks representation. Oxygen atoms are represented in red colour where as carbon atoms are represented in black colour.

In the fig. 4 the interacting amino acids with the ligands were labelled in green colour and also distances, the distances between ligand and receptor(amino acids) were shown in dotted lines. The receptor and ligand complex structure was analysed and identified the interactions in terms of Hydrogen bond and the hydrogen bond distances with their partners shown in table 2.

Protein interaction calculations

The protein interaction calculations were computed for all the proteins which were docked with estrogen receptor and ligand interactions, such as number and type of hydrogen bonds, hydrophobic interactions and aromatic side- chain interactions were calculated. The hydrogen bonds between the two interacting proteins were noted along with the interacting residues and the bond distances.

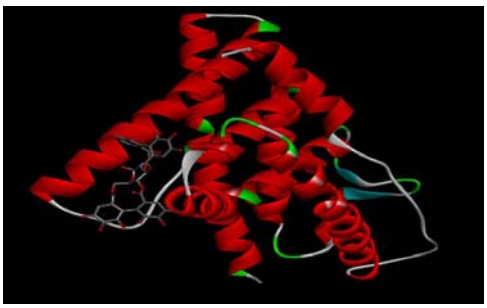


Fig. 3: Complex structure (docked) of the receptor and ligand molecule

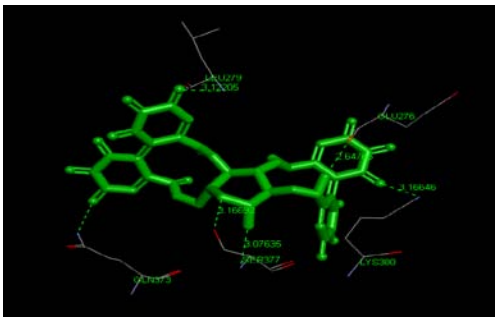


Fig. 4: Interacting residues of the Ligand with receptor molecules

DISCUSSION

Broadly speaking, In the present study we attempted to analyse the binding interactions of Pedunculagin and Estrogen receptor to know

the energy changes in the total ligand receptor complex which was found to be -108.25 Kcal /mol which is found to be a very affinity binding parameter. The structure of Pedunculagin resembles of a two bicyclic poly-hydroxyl ring structure bridged by ester linkages of D- glucose residue. The structure of Pedunculagin with its poly hydroxyl groups shows a higher surface area of contact and also greater number of hydrogen bond with the interacting sites of estrogen receptor. The ligand receptor formed 6 pairs of hydrogen bonds with a bond distance <3.20 A° with GLN373:NE2, GLU276:O as the interacting residue working at a distance of < 2.62 A° and SER377:N, LEU279:N, LYS380:NZ and SER377:OG working at distance > 2.62 A°. Taking into account the structure of Pedunculagin ligand molecule and interacting residues it infers that the overall edges surface atoms of ligand are stabilised by hydrogen bonding,which givesa idea that ligand molecule is stabilised inside the receptor active site by the oxygen atoms of hydroxyl group of ligand and ligand hydroxyl groups play a key role in these interactions. The inter molecular hydrogen bonding (-23.2086 Kcal /mole) between ligand and receptor further stabilised by vanderwaals forces of attraction which is found to be -84.8167 Kcal / mole. Both these interactions seem to be functioning by mutual synergy leading to stable ligand receptor interaction.

Table 2: Interactions in terms of Hydrogen Bond Distances

Receptor	Ligand	H-BOND Distance (A°)	Receptor Atoms (AA)	Ligand Atoms
1QKN	Pedunculagin	2.61775	GLN373:NE2	O
1QKN	Pedunculagin	2.64765	GLU276: O	O
1QKN	Pedunculagin	3.07635	SER377:N	O
1QKN	Pedunculagin	3.12205	LEU279:N	O
1QKN	Pedunculagin	3.16646	LYS380:NZ	O
1QKN	Pedunculagin	3.16692	SER377:OG	O

CONCLUSION

Selective estrogen receptor modulators (SERMs) have become the current therapeutic strategy for treatment of breast cancer. Breast cancer signalling by estrogen receptor has been delineated which up regulates angiogenesis and proliferation of tumour cells. In the current study pedunculagin is assayed using Insilco methods and found to have higher affinity for ER.

This study was conducted to show pedunculagin binding action on estrogen receptor with hydrogen bonding and vanderwaals forces playing role in ligand – receptor interactions. Further in vitro and in vivo studies on pedunculagin as a probable selective estrogen receptor modulator will prove its role in breast cancer treatment.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Katzenellenbogen, Benita S. Proliferation, hormonal responsiveness, and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. Cancer Res 1987:47(16):4355-60.
2. Ferlay J, DM Parkin, E Steliarova-Foucher. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010:46(4):765-81.
3. McGranahan, Gale, Charles Leslie. Walnuts (Juglans). Genetic Resources of Temperate Fruit and Nut Crops 290; 1991. p. 907-74.
4. Heuvel, John P, Vanden. Mechanistic examination of walnuts in prevention of breast cancer. Nutr Cancer 2012:64(7):1078-86.
5. Hyman Mark. Clinical approaches to environmental inputs. Textbook of functional medicine. Washington: Chapter Institute of Functional Medicine. 2006. p. 347-88.
6. Crozier Alan, Indu B Jaganath, Michael N Clifford. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 2009:26(8):1001-43.
7. Huang Xin, Giuseppe Mazza. Application of LC and LC-MS to the analysis of melatonin and serotonin in edible plants. Crit Rev Food Sci Nutr 2011;51(4):269-84.
8. Hardman W Elaine. Walnuts have potential for cancer prevention and treatment in mice. J Nutr 2014;144(4 Suppl):555S-560S.
9. Brown Jean K. Nutrition and physical activity during and after cancer treatment: an American Cancer Society guide for informed choices. CA Cancer J Clin 2006;56(6):323-53.
10. Reddy B Odhav, KD Bhoola. Natural products for cancer prevention: a global perspective. Pharmacol Ther 2003;99(1):1-13.
11. Ros Emilio. A walnut diet improves endothelial function in hypercholesterolemic subjects a randomized crossover trial. Circulation 2004;109(13):1609-14.
12. Accelrys Software Inc, Discovery Studio Modeling Environment, Release 2.1, San Diego: Accelrys Software Inc; 2007.
13. Bipransh Kumar Tiwary, Ravi Kant Pathak, Kiran Pradhan, Ashis Kumar Anda, Asim Kumar Bothra, Ranadhir Chakraborty. Evaluation of drug candidature of some quinazoline-4-(3h)-ones as inhibitor of human dihydrofolatereductase enzyme: molecular docking and in silico studies. Int J Pharm Pharm Sci ISSN 2014;6(2):393-400.
14. Shyam Perugu, HarikaJalli, Manjula Bhanoori. SSViewer: Sequence Structure Viewer. Int J Innovative Technol Exploring Engineering (IJITEE) ISSN 2013;3(4):2278-3075.