Journal of Pharmaceutical Research International

30(2): 1-8, 2019; Article no.JPRI.51656
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

The Antiangiogenic Effects of Tamoxifen might be Attributed to Receptor Binding Capacity of its Solvent Dimethylsulfoxide in Breast Cancer: A Molecular Docking Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ES and OK designed the study, performed the statistical analysis. Authors OK and NDT wrote the protocol and wrote the first draft of the manuscript. All authors managed the analyses of the study. Authors OK and NDT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2019/v30i230265
Editor(s):
(1) Dr. Carlos M. Contreras, Unidad Periférica Xalapa, Instituto de Investigaciones Biomédicas, UNAM, Instituto de Neuroetología, Universidad Veracruzana, Mexico.
Reviewers:
(1) Flávia Del Castanhel, Federal University of Santa Catarina, Brazil.
(2) Franco Cervellati, University of Ferrara, Italy.
Complete Peer review History: https://sdiarticle4.com/review-history/51656

Original Research Article

Received 18 July 2019
Accepted 26 September 2019
Published 12 October 2019

ABSTRACT

Aim: Tamoxifen, a Dimethyl sulfoxide (DMSO)-soluble chemotherapeutic, is widely used in the treatment of breast cancer. Tamoxifen has an anti-angiogenic effect especially on breast tumor cells by blocking VEGF(Vascular Endothelial Growth Factor) production. On the other hand, according to our previously studies, we demonstrated that DMSO could mimics the cytotoxic effects of Thalidomide in 4T1 mouse breast cancer cells and is related with the anti-angiogenic response of HeLa cells. At this point of view, the aim of this study was to determine the possible binding effects of DMSO on certain cell surface receptors.

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Methods: The *in silico* studies were implemented using the Docking Server. X-ray structures of receptor proteins' PDB files were obtained from Protein Data Bank [Human Progesteron Receptor (PDB ID: 1A28, Human Tumor Necrosis Factor Receptor-1 (PDB ID: 1EXT), Human Interferon Gamma Receptor-1 (PDB ID: 1FG9), Human Epidermal Growth Factor Receptor (PDB ID: 1IVO)]. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method.
Results: According to the molecular docking results of this study, DMSO, the general solvent of Tamoxifen, has a 90% binding capacity to the Interferon Gamma (IFNɣ) receptor and 100% to Tumor Necrosis Factor alfa (TNFα) receptor, respectively.
Conclusion: These receptors have significant effects on the proliferation and angiogenesis of cancer cells which leads to the metastasis of breast cancer. In conclusion, the antiangiogenic effects of Tamoxifen might be reduced due to its solvent DMSO's angiogenic effects.

Keywords: Tamoxifen; DMSO; IFNɣ; TNFα; molecular docking; angiogenesis; breast cancer.

ABBREVIATIONS

ALA : Alanine
ARG : Arginine
ASN : Asparagine
ASN : Aspartic acid
CYS :Cysteine
GLN : Glutamine
GLU : Glutamic acid
GLY : Glycine
HIS : Histidine
ILE : Isoleucine
LEU : Leucine
LYS : Lysine
MET : Methionine
PHE : Phenylalanine
PRO : Proline
SER : Serine
THR : Threonine
TRP : Tryptophan
TYR : Tyrosine
VAL : Valine

1. INTRODUCTION

Breast cancer is the primary reason for cancer-related death in women worldwide [1]. Female sex hormones show significant effect on the induction of breast cancer. One of the most important female sex hormone is estrogen. Estrogen binds its receptor (Estrogen Receptor: ER) and then triggers certain signaling pathways that results in progression and reoccurrence of breast cancer [2].

Tamoxifen, a nonsteroidal antiestrogen, is widely used in the treatment of estrogen receptor (ER) positive breast cancer. It has been determined that usage of tamoxifen could cause a decrease recurrence and mortality of breast cancer as 50% and 30% respectively [3].

According to Sigma Aldrich product information page, an anti-angiogenic drug Tamixofen is water-insoluble and an organic solvent should be used in order to solve this drug. The most important organic solvents are methanol, ethanol, 2-propanol, propylene glycol and dimethyl sulfoxide (DMSO) [4].

There are certain DMSO-solved tamoxifen applications in both *in vitro* and *in vivo* studies Generally, Tamoxifen could lead an cytostatic antiproliferative and anti-tumoral as well as antifungal effects in *in vitro* studies [5,6,7,8,9] According to our previously articles we showed that DMSO could mimic the cytotoxic effects of Thalidomide on murine breast cancer cells [10] and suppressed HeLa cell proliferation [11].

In this study, our main aim is to determine the possible binding effects of DMSO and tamoxifen on cell surface receptor proteins which are important for the progression of breast cancer metastasis. The chosen- surface receptor proteins are; HER2 (Human Epidermal Receptor-2), IFNɣ R-1 (Interferon Gamma Receptor-1), PR (progesterone receptor), TNFα Receptor (Tumor necrosis Factor alfa receptor).

According to docking studies that we performed DMSO has a 100%, 90%, 70% and 50% binding frequency to TNFα R, IFNɣ R1, HER2 and PR receptors respectively. We have also determined the binding frequencies of Tamoxifen to the same receptors. Based on these results, tamoxifen shows 50%, 40%, 20% and 50% binding frequencies to TNFα R, IFNɣ R1, HER2 and PR receptors respectively. It seems that DMSO could affect the anti-angiogenic properties of Tamoxifen. Especially for TNFα receptor, binding capacity of DMSO is greater than Tamoxifen itself. It is accepted that these

receptors have significant effects on the proliferation and angiogenesis of cancer cells. These receptors lead to the metastasis of breast cancer. Thus, DMSO in which binds to these receptors might change the anti-angiogenic effects of Tamoxifen for at least in breast cancer.

2. MATERIALS AND METHODS

This research was carried out in the research laboratory of the Faculty of Health Sciences at Akdeniz University in Antalya/TURKEY. The *in silico* experimental process took about one month.

The *in silico* studies were implemented using the Docking Server according to Bikadi and Hazai, 2009 [12].

2.1 Protein Structure Preparation

The X-ray crystal structures of receptor proteins were obtained from Protein Data Bank. PDB file names are; PDB ID: 1A28; PDB ID: 1EXT; PDB ID; PDB ID: 1FG9; PDB ID: 1IVO for Human Progesteron Receptor; Human Tumor Necrosis Factor Receptor-1 ; Human Interferon Gamma Receptor-1; Human Epidermal Growth Factor Receptor respectively. Protein Preparation of the Docking Server was carried out to clean the protein, select the PDB Box and determine the protein properties [13,14,15,16].

2.2 Ligand Structure Preparation

The structures of dimethyl sulfoxide (DMSO) and tamoxifen were used in Docking Server. MMFF94 method was used to Geometry optimization and Gasteiger method was used to calculate charge in pH 7.0. DMSO ligand properties were determined as; molar weight: 79.142, molpol: 8.04, MMFF94 energy: 3.87134 kcal/mol. Tamoxifen ligand properties determined as; molar weight: 372.523, molpol: 46.51, MMFF94 energy: 110.32534 kcal/mol.

2.3 Molecular Docking

Docking calculations were done by using Docking Server [12]. Gasteiger partial charges were inserted to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were identified.

Docking calculations were performed for both tamoxifen and DMSO. According to the aid of AutoDock tools, the essential hydrogen atoms, Kollman united atom type charges and solvation parameters were inserted [17]. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Docking simulations were done by using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [18]. Initial position, orientation, and torsions of the ligand molecules properties were randomly set. According to the docking procedure, all rotatable torsions were released. Each docking experiment was gained from 10 different steps that were set to finalize after reaches to 250000 energy evaluations. 0.2 Å translational step and 5 torsion steps were applied during the searching proses.

3. RESULTS AND DISCUSSION

DMSO and Tamoxifen binding frequencies and binding energies to four different receptors summarized in Table 1 and Table 2 respectively. The possible hydrogen bonds and the amino acids between ligands and receptor proteins are shown in Table 3.

Table 1. Binding frequencies of DMSO to four different receptor proteins

Receptor name	Estimated free energy of binding	Estimated inhibition constant, Ki	vdW/Hbond/ desolv energy	Electrostatic energy	Total intermolec. energy
HER2 (1IVO)*	-1.89 kcal/mol	40.91 mM	-1.82 kcal/mol	-0.07 kcal/mol	-1.89 kcal/mol
IFNγR (1FG9)*	-2.11 kcal/mol	28.42 mM	-2.07 kcal/mol	-0.04 kcal/mol	-2.11 kcal/mol
TNFαR (1EXT)*	-2.05 kcal/mol	31.30 mM	-1.92 kcal/mol	-0.13 kcal/mol	-2.05 kcal/mol
PgR (1A28)*	-2.48 kcal/mol	15.27 mM	-2.45 kcal/mol	-0.03 kcal/mol	-2.48 kcal/mol

**PDB (Protein Data Bank Code)*

Table 2. Binding frequencies of tamoxifen to four different receptor proteins

Receptor name	Estimated free energy of binding	Estimated inhibition constant, Ki	vdW/Hbond/ desolv energy	Electrostatic energy	Total intermolec. energy
HER2 (1IVO)*	-4.27 kcal/mol	753.38 μM	-5.27 kcal/mol	-0.35 kcal/mol	-5.63 kcal/mol
IFNγR (1FG9)*	-6.39 kcal/mol	20.65 μM	-7.85 kcal/mol	+0.17 kcal/mol	-7.68 kcal/mol
TNFαR (1EXT)*	4.54 kcal/mol	466.66 μM	-5.94 kcal/mol	-0.08 kcal/mol	-6.03 kcal/mol
PgR (1A28)*	-7.07 kcal/mol	6.55 μM	-9.22 kcal/mol	-0.09 kcal/mol	-9.31 kcal/mol

**PDB (Protein Data Bank Code)*

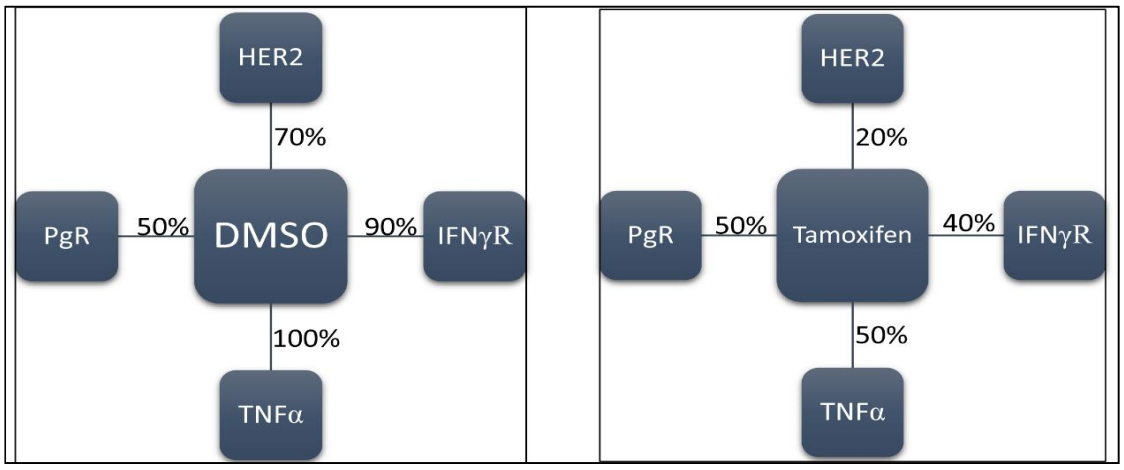

Diagram 1

The 3-D interaction sites and binding frequencies of ligands and receptors are given in the Diagrams 1 and 2.

Breast cancer is a complex disease which has a high frequency in women. It is still the second leading cause of cancer mortality and accounting for 14% of the all cancer related deaths in worldwide [9]. Hormones play a significant role in breast cancer. According to Kim J-Y et al., 2018, European Society of Medical Oncology (ESMO) and the American Society of Clinical Oncology (ASCO) agree with the fact that the usage of endocrine therapy is better than cytotoxic chemotherapy for metastatic breast cancer patients [19].

Tamoxifen widely used in breast cancer treatment is an estrogen receptor antagonist especially for hormone-responsive patients. It is generally dissolved in Dimethyl sulfoxide (DMSO). In fact, according to our previously studies DMSO could affect and/or mimic the cytotoxic effects of chemotherapeutics *in vitro*. Based on these results, we aimed to investigate the possible binding properties of DMSO on certain types of receptors which are often responsible for the aggressiveness of the breast cancer [7].

According to the progression in breast cancer pathogenesis, it has been found that hormone-based signaling pathways as well as their receptors are crucial in breast cancer and breast cancer-targeted hormone therapy plays an important role in clinical treatment [20]. The main receptors regarding in breast cancer are HER2, IFNɣR, TNFαR and PR.

Human epidermal growth factor receptor HER2 is overexpressed about 20–30% of breast cancer patients and related with a more aggressive phenotype that resulted in a short life time [21]. There is no identified specific

ligand for the HER2 receptor extracellular domain. It has been suggested that HER2 is dimerized with other EGF receptors in a ligand independent wayand gains its active confirmation [22]. In our study, the solvent of tamoxifen DMSO could bind the HER2 receptor with a 70% frequency while the tamoxifen itself could bind the same receptor with a 20% frequency. We suggested that the solvent DMSO could trigger the HER signaling pathway when DMSO-Solved tamoxifen used in a clinical way.

We have chosen the Progesterone receptor (PgR) is a second one to determine the possible binding effects of DMSO. It is generally used to determine the aggressiveness of the breast tumors. It has been reported that patients with a high PgR expression is related with a weak prognosis. According to our molecular docking results, both DMSO and Tamoxifen could bind the PgR with the same frequency as 50%. So we could assume that DMSO could mimic the PgR binding properties of Tamoxifen.

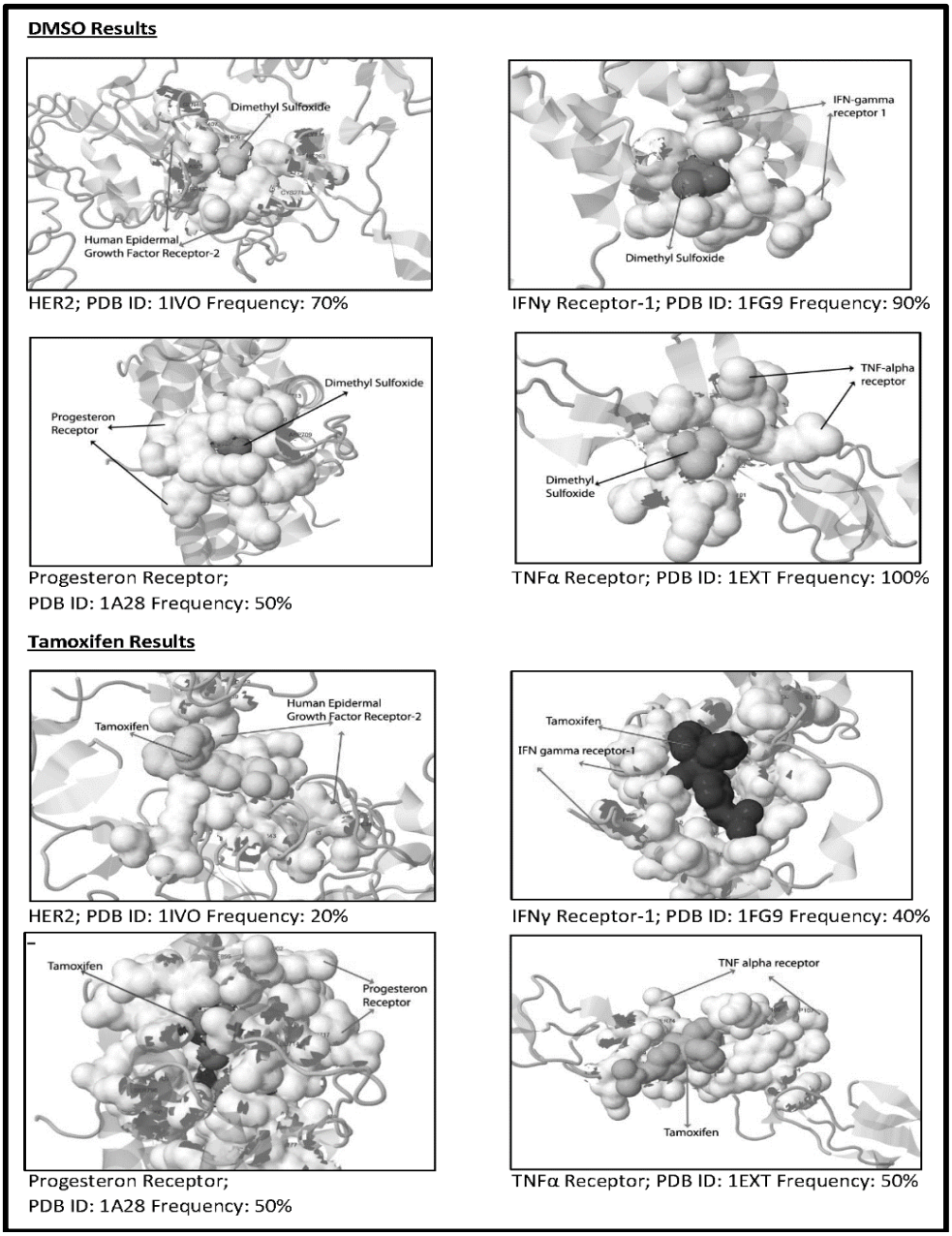

Diagram 2

Table 3. Residues of receptor proteins that interact with DMSO and Tamoxifen

Receptor name	Interactions DMSO		Interactions tamoxifen		
HER2	263: PHE	275: TYR	8: GLN	344: ASP	406: THR
(1IVO)*	272:PRO	407: LYS	38:LEU	346: HIS	407: LYS
	274: ASN	434: ASP	285: ARG	380: PHE	
IFNγR	54: PHE	61: LYS	8: ALA	49: ILE	76: ASP
(1FG9)*	57: PHE	74: LYS	11: LEU	53: TYR	77: MET
	58: LYS		33: LEU	73: ILE	80: LYS
TNFαR	98: CYS		74: SER	95: VAL	81: PHE
(1EXT)*	104: ARG		82: GLN	96: CYS	106: TYR
			93: ASP	104: ARG	112: PHE
PgR	711: SER	791: GLU	715: LEU	759: MET	890: TYR
(1A28)*	714: LEU	794: PHE	718:LEU	760: VAL	891: CYS
			719: ASN	763: LEU	903: VAL
			721: LEU	778: PHE	905: PHE
			725: GLN	801: MET	909: MET
			756: MET	887: LEU	

**PDB (Protein Data Bank Code)*

The multifunctional cytokines IFN-γ and TNF-α are accepted as the significant factors which are found to play important role in apoptosis and cancer as well as in inflammation and immunity [23]. IFN-γ potentially activates caspases and is strongly disturbed in invasive breast tumors. That is why breast cancer cells could easily metastases in the late stages of breast cancer. TNF‑α interacts on a synergistic way with IFN‑γ. They have been shown to induce apoptosis by activating of the caspase signaling in many cancer cells types especially for breast cancer [24].

As it is accepted that immunity is very important for cancer progression, we have chosen the Interferon gamma (IFN-γ) and TNF- α receptors as the other receptors for this study. It has been found that DMSO has a great potential for binding to these receptors with a very high frequencies, 90% and 100% respectively as compared with Tamoxifen's binding frequencies as 40% and 50% respectively.

This is the first report showing that the solvent of Tamoxifen, DMSO has a great binding potential to especially for IFN-γ and TNF-α receptors. It must be kept in mind that the solvents of the drugs could be as effective as the drugs themselves.

4. CONCLUSION

In conclusion, these receptors have significant effects on the proliferation and angiogenesis of cancer cells which leads to the metastasis of breast cancer. In particular, the data obtained from the computer-based approaches figure out how important effects do have the signaling pathways related with cancer. However, In conclusion, the antiangiogenic effects of Tamoxifen might be reduced due to its solvent DMSO's angiogenic effects.

The most important limitation of such kind of studies is that these are just done *in silico*. *in vitro* experiments should be done to come to a significant knowledge. Further studies must be needed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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