



# The study of drug resistance properties of ABCG2 (ATP-binding cassette G2) in contact with thymoquinone, gallic acid, and hesperetin antioxidants

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## ARTICLE INFO

### Article Type:

Original Article

### Article History:

Received: 12 May 2018

Accepted: 24 December 2018

### Keywords:

Antioxidant

ABCG2 transporter

Cancer cell

Simulation

## ABSTRACT

**Introduction:** ATP-binding cassette (ABC) transporters are a group of intra membrane proteins that play key roles in the transmission and exchange of vital compounds on both sides of the membrane. These proteins can specially transport anti-cancer drugs out of cancer cells. ABCG2 is a member of this family that is extremely expressed in many cancers. This study, aims to evaluate the binding affinity of three antioxidants thymoquinone (TQ), gallic acid (GA), and hesperetin (HP) to ABCG2 compared with an anti-cancer drug, mitoxantrone (Mit), to export cells.

**Methods:** The PDB file of ABCG2 was obtained from the protein data bank server (<http://www.rcsb.org>) with ID: 5NJ3. After 200 stages of molecular docking running on ABCG2 protein in AutoDock v.4.2 software, the amino acids involved in the binding site of each compound were identified using the LigPlot<sup>+</sup> software.

**Results:** HP had the lowest (-6.36 kcal/mol) and GA had the highest (-3.93 kcal/mol) binding energy in comparison with Mit (-0.06 kcal/mol) for binding to ABCG2. Effective concentration required to perform the reaction between ABCG2 was higher in GA (1.31 mM) than TQ (42.69  $\mu$ M) and HP (21.74  $\mu$ M). GA, HP, and TQ formed 17, 18, and 22 hydrogen and hydrophobic bonds at the binding site of ABCG2.

**Conclusion:** It seems that GA has the lowest affinity to make contact with ABCG2 binding site. So, GA tends to remain in the cell but TQ and HP tend to leave the cell easily via ABCG2 transporter.

### Implication for health policy/practice/research/medical education:

Gallic acid is an antioxidant that has the low tendency for expose out of the cells. It as an effective antioxidant is better than TQ and HP for treatment of cancers.

*Please cite this paper as:* Saffari-Chaleshtori J, Shafiee SM, Ghatreh-Samani K, Jalilian N. The study of drug resistance properties of ABCG2 (ATP-binding cassette G2) in contact with thymoquinone, gallic acid, and hesperetin antioxidants. J Herbm Pharm. 2019;8(2):108-113. doi: 10.15171/jhp.2019.17.

## Introduction

ATP-binding cassette (ABC) transporters are a family of proteins that are present in cell membrane and play key roles in the transport of vital compounds out of the cells (1). There are 48 ABC transporter proteins, known in human body cells, which are divided into seven subgroups of ABC (A-G) based on their size, number of subunits and their building (2,3). Any malfunctioning of these membrane proteins causes a variety of diseases and the occurrence of mutations in the building of these molecules also causes

genetic disorders such as cystic fibrosis (4), gout (5) and lipid metabolic abnormalities (6). Today, the importance of these membrane transporters has increased due to their role in resistance to anticancer drugs that cause the drug to escape from the cell before it exerts its effect (7). ABCG2 transporters are of the most important members of this great family. They exist and have physiological roles in many tissues, such as breast, brain, and testicular, as well as in many cancers, especially breast cancer, stomach cancer, and pancreatic cancer (8-10). The most important

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physiological role of ABCG<sub>2</sub> transporter is the transfer of uric acid. Any disruption in the normal functioning of this protein causes diseases such as gout, hypertension, and kidney disorders (11). In addition, its expression is increased in many cancers and can be recognized as a marker of cancer at the cell surface. However, it is a proprietary marker for hematopoietic stem cells (12). ABCG<sub>2</sub> as an intracellular drug transporter, causes the drug resistance in cancer cells (13). Despite significant advances in the treatment of various types of cancers, there is still an increasing incidence of cancer in different societies (14,15). On the other hand, studies have shown that herbal compounds play a very important role in the death of different types of cancer cells (16). Hesperetin (HP) or 2,3-Dihydro-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1 benzopyran-4-one), gallic acid (GA) or 3,4,5-trihydroxybenzoic acid and thymoquinone (TQ) or 2-Isopropyl-5-methylbenzo-1,4-quinone (17,18) are effective compounds found in plants such as onion, apple, green and black tea, red grapes, citrus, broccoli sprouts, tomato (19) and in some fruits like berries and grapes, hard wood plant species such as chestnut (*Castanea sativa* L.) and oak (*Quercus robur*), and also in tea (20) and *Nigella sativa* Linn (21). Many studies have shown that these three active plant compounds are very effective in destroying cancer cells, especially gastric cancer cells (22-24). Because of the importance of ABC transfer proteins in cancer cells, especially gastric cancer cells, as well as the confirmation of the effects of HP, GA, and TQ antioxidants on cell death in gastric cancer cells. This study, was designed to evaluate the effects of these three antioxidants on ABCG2 transporter using simulation and molecular docking and measuring the tendency of this protein to remove these three plant compounds from the cell, compared to anticancer drug, mitoxantrone (Mit).

## Materials and Methods

We obtained the ABCG2 PDB file (ID: 5NJ3) from protein data bank server (<http://www.rcsb.org>) and optimized with Arguslab v.4.0 software. Then, we obtained the antioxidant files of GA (CID:370), HP (CID: 72281), and TQ (CID:10281) from Pubchem server and converted them to PDB files and optimized using Arguslab v.4.0 software (25).

Molecular docking studies were done by Autodock v.4.2 software in Linux operating system by a 64-bit system with Intel® Core™ i7 CPU Server in Clinical Biochemistry Research Center, Basic Health Sciences Institute of Shahrekord University of Medical Sciences.

Molecular docking of GA, HP, and TQ on ABCG2 transporter was done to find the best binding sites for the ligand-receptor and to determine the most stable free energy state of ligand-receptor. In this study, we built a Grid Box with 60×120×72 nm (x×y×z) for intra cellular NBD domains, after the production of PDBQ and PDBQT

files of TQ, GA, and HP as ligands and ABCG2 transporter as receptor. We used *autogrid 4 -p n.gpf -l n.gle* linux in order to produce the n.gle text file. After 200 stages of molecular docking running on ligands, we used genetic algorithm (GA) and Lamarckian GA parameters. To produce the n.dlg text file, we used *autodock 4 -p n.dpf -l n.dlg* linux order. The data obtained from n.dlg file, were analyzed (26).

In this study, we used LigPlot<sup>+</sup> software to specify the number of hydrophobic and hydrogen bonds between ABCG2 transporter and GA, TQ, and HP ligands and the type and number of amino acids present in the binding site were identified (27).

## Results

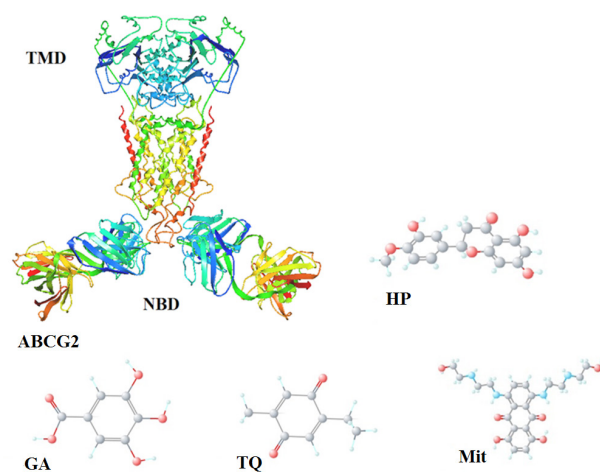
Figure 1 shows the structure of ABCG2 transporter and three antioxidants HP, GA, and TQ. In this figure, nucleotide binding domains (NBDs) in intra cellular membrane and transmembrane domains (TMDs) in extra cellular membrane were visible.

Table 1 shows the molecular interaction and the binding energies of Mit, GA, HP, TQ, with ABCG2. After Mit, the estimated binding free energy (BE) for GA was higher than HP and TQ. Estimated inhibition constant (EIC) for GA was lower than HP and TQ.

Figure 2 shows that the interaction between ABCG2 with TQ has the most hydrogen and hydrophobic bonds and after the interaction between ABCG2 with Mit, interaction between ABCG2 with GA has the least hydrogen and hydrophobic bonds.

## Discussion

The potential of stem cells in the tissues and the ability to differentiate into somatic cell types have played very important roles in the reconstruction of damaged tissue parts (28,29). Cancer stem cells (CSCs), which in many



**Figure 1.** The structure of molecules. ABCG2 transporter with nucleotide binding domains (NBDs) and transmembrane domains (TMDs). HP: hesperetin, GA: gallic acid, TQ: thymoquinone, Mit: mitoxantrone.

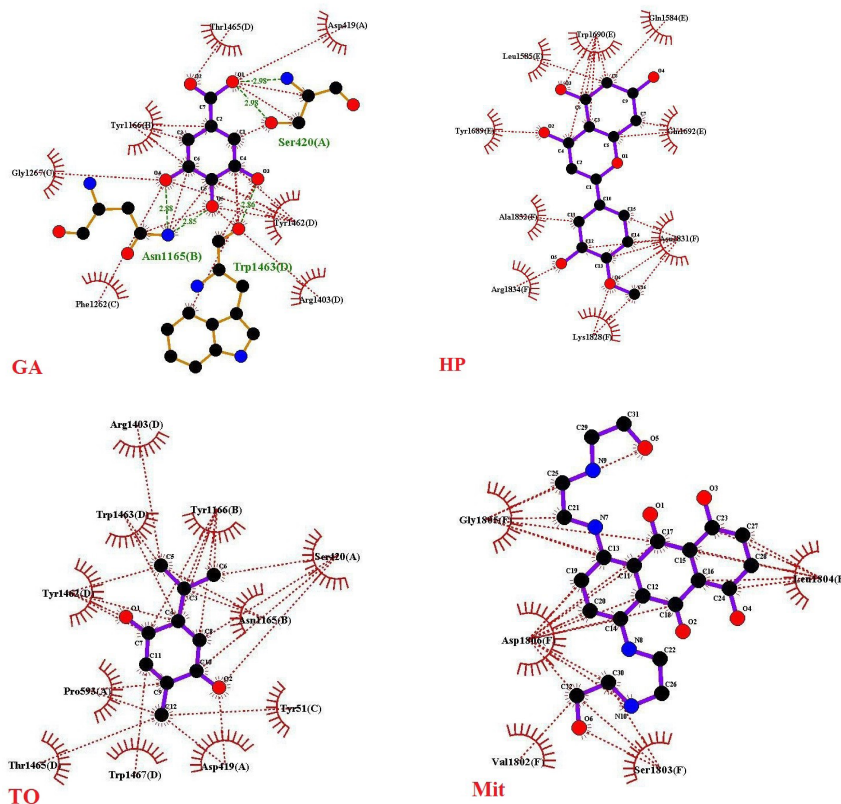
**Table 1.** Molecular docking parameters between ABCG2 transporter and other ligands

Interaction bonds		EIC	FIE	BE	Ligands
Hydrophobic Bonding	Hydrogen Bonding				
Val1802, Ser1803, Leu1804, Gly1805, Asp1806	Val1802, Ser1803, Leu1804, Gly1805, Asp1806	900.1 mM	-4.48	0.06-	Mit
Asp419, Tyr1462, Arg1403, Phe1262, Gly1264, Tyr 1166, Thr1465	Ser420, Trp1463, Asn1165, Asp419, Tyr1462, Arg1403, Phe1262, Gly1264, Tyr 1166, Thr1465	1.31 mM	-5.72	-3.93	GA
Gln1584, Gln1692, Asn1831, Lys1828, Arg1834, Ala1832, Tyr1689, Leu1585, Trp1690	Gln1584, Gln1692, Asn1831, Lys1828, Arg1834, Ala1832, Tyr1689, Leu1585, Trp1690	21.74 $\mu$ M	-6.96	-6.36	HP
Tyr51, Ser420, Asn1165, Asn419, Trp1467, Thr1465, Pro593, Tyr1462, Trp1463, Arg1403, Tyr1166	Tyr51, Ser420, Asn1165, Asn419, Trp1467, Thr1465, Pro593, Tyr1462, Trp1463, Arg1403, Tyr1166	42.69 $\mu$ M	-6.26	-5.96	TQ

Abbreviations: BE, Estimated Free Energy of binding (kcal/mol); FIE, final intermolecular energy (kcal/mol); EIC, estimated inhibition constant; Mit, mitoxantrone; GA, gallic acid; HP, hesperetin; TQ, thymoquinone.

cases originate from stem cells, also behave like stem cells. They have the ability to grow, replicate, and differentiate into other cancer cell lines (30-33). However, studies have shown that, at the CSCs surface, cell-specific markers increase expression, which can be specific for each cell line. For example, in GCSC cells, ABCB1 cell markers, CD133, ABCG2, are strongly expressed (9). Increase in expression of ABC carriers induces a unique feature in GCSC cells, which makes them resistant to chemotherapy (34). Nowadays, the structure and function of ABC transporter

proteins are well known. This family of proteins consists of two domains, NBDs with ATPase activity inside the cell and two TMDs attached to the outer membrane (35,36). Our study well illustrated that the ABCG2 transporter had a great ability to convey different compounds out of the cell. The strong tendency of GA, TQ, and QC toward the binding site in the NBD domains represents a tendency for this transition. Recent studies have shown that the amino acid E446 in NBD domain of ABCG2 transfer, plays an important role in the transfer of drugs to the outside of



**Figure 2.** Interaction bonds such as hydrogen and hydrophobic bonds between ABCG2 with Mit, GA, HP and TQ. Mit, mitoxantrone; GA, gallic acid; HP, hesperetin; TQ, thymoquinone.

the cell as well as the ATPase property of this transporter. So that the removal of any of the E466K, E451R, E458R D477R amino acids causes a disruption of protein function and the deficiency of efflux of the drug Mit from cell. Also, K647, K652, K653 amino acids in the TMD domain interact with cellular membrane phospholipids electrostatically, which stabilizes the protein and binds to the cell membrane. However, they don't have a significant role in the efflux of Mit drug (37). Other studies have shown that the amino acid K473 plays an important role in the formation and deployment of monomers alongside each other (38-40). The ABCG2 transporter plays an important role in the efflux of cholesterol from the cell. Taylor et al. showed that six amino acids F432, F439, L539, I543, V546, and M549 play a key role in connecting two cholesterol molecules to the ABCG2 molecule (41). The fluorescent dyes Hoechst 33342 and rhodamine123 (R123) are desired substrates for the ABCG2 and ABCB1 transporters, respectively (42). In our study, Figure 2 and Table 1 show that anti-drug cancer Mit, just interacted with five amino acids (Val1802, Ser1803, Leu1804, Gly1805, Asp1806) in binding site. However, the highest amount of estimated binding free-energy (BE) was -0.06 kJ/mol and the highest EIC was 900.1 mM. This indicates that the rate at which Mit binds to the binding site in the ABCG2 molecule is very low and the possibility of efflux of this drug from the cell by this transporter is very low. In addition, in order to allow this connection to be thermodynamically applicable, the Mit concentration of the cell should reach 900.1 mM. This concentration of drug is very high. However, at much lower concentrations, the drug can play its role in the treatment process in cancer cells. Many studies have shown that GA is a powerful antioxidant compound in the death of cancer cells (43,44).

Based on the results in Figure 2 and Table 1, GA interacts with amino acids in binding site by ten hydrogen bonds and six hydrophobic bonds. Its BE was -3.93 kcal/mol and its EIC was 1.31 mM. These amounts are lower than the amounts obtained from Mit connection with the binding site. However, compared to the two anti-oxidants, HP and TQ are much higher, such that HP and TQ had 18 and 22 interaction bonds with ABCG2 and their BE were -6.96 and -6.36 kcal/mol. HP and TQ with lower concentrations of 21.74 and 42.69  $\mu$ M were in contact with ABCG2. This indicates that they tend to efflux the cell. Previous studies have shown that the IC<sub>50</sub> concentrations of these three antioxidant compounds are different for the induction of anticancer effects in different cancer cells. The lowest IC<sub>50</sub> has been obtained in comparison with HP and TQ for the GA molecule (25,45,46). This can be a reason for the results obtained in this study. Our study showed that GA compared to other two compounds could be more resistant to low concentrations in cancer cells, hence, it exerts its anticancer effects, while two other drugs (TQ and HP) need to use much higher concentrations, which

can have toxic effect on other cells.

## Conclusion

Although antioxidant compounds can inhibit cancer cells and induce cancer cell death, but proprietary transporters, such as ABCG2, discard these compounds and other anti-cancer drug compounds from cancer cells. This study showed that GA, compared with TQ and HP by a very low binding affinity with these membrane transfer proteins, could have longer existence in the cell, and would have a higher effect on the removal of cancer cells.

## Authors' contributions

SMS and KGS designed and managed the study. JSC and NJ carried out the molecular dynamic studies. JSC prepared the draft. All authors read and approved the final version of the manuscript.

## Conflict of interests

Authors declare that there is not any conflict of interest.

## Ethical considerations

Ethical issues including text plagiarism, misconduct, manipulation or appropriation, data fabrication, falsification, redundant publication as well as duplicate submissions have been carefully observed by authors.

## Funding/Support

This study was funded by the Deputy of Research and Technology of the Shahrekord University of Medical Sciences, Shahrekord, Iran, based on the research project number: 267.

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