Research Article

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Journal of In Silico & In Vitro Pharmacology
ISSN 2469-6692+non

2017
Vol.3 No.3:21

DOI: 10.21767/2469-6692.100021

Garlic Treatment to Brain Cancer: An *In-silico* Evaluation to Explore the Therapeutic Efficacy of Allicin by Inhibition of Brain Aquaporin

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Received date: Jul 09, 2017; **Accepted date:** Jul 20, 2017; **Published date:** Jul 27, 2017

Citation: Verma S, Pandey AK (2017) Garlic Treatment to Brain Cancer: An *In-silico* Evaluation to Explore the Therapeutic Efficacy of Allicin by Inhibition of Brain Aquaporin. J In Silico In Vitro Pharmacol. Vol 3. No 3:21

Abstract

Background: Brain cancer prevalence is increasing at an explosive pace. Aquaporins (AQPs) the integral membrane water channel proteins are diversely distributed in body and maintain osmotic gradient across cell's plasma membrane. Past researches proved that AQP1, AQP4, AQP5 and AQP9 are majorly found in brain and are overexpressed in malignant conditions elevating progression, metastasis, angiogenesis, and associated edema. Allicin a plant origin compound found in garlic (*Allium sativum*), has anticancer, antioxidant, anti-inflammatory, antifungal and antibacterial properties. Although much literature is present for allicin in treating cancer, inhibition of AQPs in brain cancer has never caught much attention. In this study we performed molecular docking to reveal structural inhibition of AQP1, 4, 5 and 9 by allicin and also analysed molecular and ADMET properties and bioavailability scores of allicin for its consideration as target specific drug.

Methods and findings: Molecular docking was performed for analysing inhibition potential of allicin for AQP1, 4, 5 and 9 by using Autodock 4.0. The ADMET properties were analysed using Swiss ADME and Protox web portal. The molecular properties and bioavailability were checked by Molinspiration web based tool. Our results of docking for AQP1, AQP4, AQP5 and AQP9 with allicin gave binding energies of -4.76 kcal/mol, -4.89 kcal/mol, -4.19 kcal/mol and -4.78 kcal/mol respectively with 1 hydrogen bonds in each interaction.

Conclusion: Brain aquaporins inhibition by allicin can be a potent target specific treatment for brain cancers.

Keywords: Aquaporin; Allicin; Brain cancer; Molecular docking

Abbreviations:

AQP: Aquaporin; ADME: Absorption, Distribution, Metabolism, Excretion; LD50: Lethal dose 50; RCSB: Research Collaboratory for Structural Bioinformatics; BCECs: Brain Capillary Endothelial Cells

Introduction

Brain cancer being the most fatal one of all types of cancer is increasing its victims exponentially. The most vulnerable population belongs to North America, Western Europe and Australia. Out of all kind of brain cancers gliomas are the most prevalent one and accounts for 70% of cases [1]. Although brain edema is common to all types of brain injury but plays most encouraging role in brain cancer progression. Peritumoral edema is characterized to be present in brain tumors and this edema result from the leakage in microvasculature around tumor which permits entrance of fluid into parenchyma of brain form microvascular lumen [2].

An exclusive family of transmembrane proteins called Aquaporins (AQPs), present in diverse cell types accounts for water transport to maintain the osmotic gradient across the plasma membrane of cells [3]. They also play an integral part in transport of some solutes like glycerol, ions and gases. In total, human AQPs constitute a group of 13 members, which are present as water transport AQPs like AQP1, 2, 4, 5 and 8 and water and solute transporters as AQP3, 7, 9 and 10. Also AQP1, 4 and 5 have permeability for ion and gas flow [4]. In normal conditions, these AQPs contribute to in maintain water homeostasis in brain, concentration of urine, secretion of exocrine gland, metabolism of fat, moistening of skin and signal transduction in neurons [5]. The AQP composition of brain constitutes AQP1, AQP4, AQP5 and AQP9 and past researches are evident for overexpression of these AQPs during malignant conditions [4,6].

AQP1, the first member of aquaporin family finds its place of expression in choroid plexus epithelium and is known to act in cerebrospinal fluid formation [7]. In diseased state, like

astrocytomas, AQP1 was reported to be overexpressed in neoplastic astrocytes and endothelia of microvessel, and in metastatic carcinomas AQP1 was showing enhanced expression in reactive astrocytes and endothelia of microvessels [8]. Increased levels of AQP1 in astrocytomas of high grade was proved by immunohistochemistry, western blot, differential gene expression, reverse transcriptase PCR and cDNA gene array analysis [9,10]. Additionally association of AQP1 expression and BCECs (Brain Capillary Endothelial Cells) in brain cancer provided support to the fact that AQP1 increase is responsible for vasogenic edema. As it is a known fact that AQP1 is not expressed by BCECs in normal brain [4]. Another research observed that AQP1 polymorphism at 783 G/C supports extension of survival in glioblastomas proving the role of normal AQP1 in enhancing glioblastomas [11]. AQP1 was also reported to be upregulated in subependymomas. Also AQP1 was present in the dura invading meningioma capillaries and cells and also its presence was detected in association with Na-K-2Cl cotransporter (ionic transporters found in different kind of brain lesions to affect movement of fluid) [12]. Thus role of AQP1 is well established in developing brain edema, growth of tumor, neoplastic astrocytes invasiveness and angiogenesis.

AQP4, majorly expressed in foot processes of perimicrovessel astrocytes of brain (in lined with endothelial cells of vessels) and also in the ependymal cell's basolateral membrane, plays essential role in maintaining water homeostasis by regulating the permeability of water across blood brain barrier [4,5]. There are many evidences from past researches of overexpression of AQP4 in tumorogenic state thus causing increase in permeability of brain tissue resulting in edema [7]. Almost all brain tumors have great association with edema including metastatic adenocarcinomas and gliomas. In human, increase of AQP4 was prominent in edema concerned with astrocytomas and pronounced correlation of this increase with enhanced breakdown of blood brain barrier was documented [10]. These finding made it evident that AQP4 levels have great contribution in enhancing edema [13]. In a past research less brain swelling was reported in mice AQP4 gene knockout model [14]. The post-effects of edema are enhanced intracranial pressure, disrupted homeostasis of tissues and hampered local blood supply causing neurologic disturbance resulting in apoptosis in neurons [14]. Also some reports established that AQP4 has great role in enhancing the progression, invasion and metastasis in brain tumor [14].

AQP5 is the least studied AQP in context of brain cancers and is known to be present in the plasma membrane and cytoplasm of astrocytes. There are also evidences for its expression in neurons [15,16]. Experiments performed by incorporating AQP5 promoter polymorphism studies concluded reduction in edema proving that there is over expression of AQP5 in meningioma patients increasing the intensity of peritumoral edema [15]. AQP5 was also reported to have concerns with Ras signalling pathway responsible for growth induction in tumorogenic conditions. In another research, increased levels of AQP5 were also reported in the astrocyte hypoxic injury enhancing migration and process elongation of astrocytes [17].

AQP9, another member of aquaporin family is not only responsible in water transport but also in solute transport like lactate, β-hydroxybutyrate, glycerol, pyrimidines, purines, and urea [18]. Its expression in brain is diversified including astrocytes and catecholaminergic neurons [18]. In past research on glioblastoma, AQP9 expression was reported in astrocytes showing malignancy and in Calgranulin B^+ cells and $CD15^+$ cells working for tumor infiltration [18,19]. Also in some other previous researches on glioblastoma overexpression of AQP9 was reported. Another study reported increase in grade of astrocytoma in immunoblots with the increase in levels of AQP9 [19]. Differential expression of AQP9 was reported, as AQP9 are found to have limited expression in normal condition of brain but diversified expression was noticed in glioblastoma concluding that AQP9 need signals from perivascular tumors or they may be limited to glioma progenitor stem cells [20]. In an immunohistochemical study immunoreactivity of anti-AQP9 was noticed to be elevated in glioma cells of glioblastoma thus suggesting elevation in levels of AQP9 which may contribute to energy metabolism of glioma cells and surrounding neurons by providing glycerol [18,20].

In light of all the above findings we considered AQP1, AQP4, AQP5 and AQP9 as the major role playing proteins in the progression of brain cancers. Hence, we considered them as effective and potent targets for the treatment of brain cancer in our present study.

Allicin (S-Prop-2-en-1-yl prop-2-ene-1-sulfinothioate) an organosulphur compound found in garlic (*Allium sativum* of family *Amaryllidaceae*), is well established for its antimicrobial, antioxidant, anti-inflammatory and anticancer properties [21,22]. As oxidative stress and inflammation are common pathology of all types of cancer the anti-oxidative and anti-inflammatory properties of allicin also supports in this context along with its anticancer properties [22]. Effect of allicin in colon, stomach, pancreas, esophagus and breasts cancers is well documented by inhibition of various pathways. Down regulation of VEGF (Vascular Endothelial Growth Factor), uPAR (urokinase receptors) and HPA (Helix promatia lectin) in colon cancer was noted in experiments with application of Allicin. Inhibition of TNFα thus reducing VCAM-1 expression via pathways of ERK1/2 and NFδB in breast cancer was also observed as an effect of Allicin [23]. But inhibitory effect of allicin on aquaporins in brain cancer is not yet researched. So, to go in depth of this context we performed molecular docking study to look for any structural inhibition of AQP1, AQP4, AQP5 and AQP9 by allicin. We also worked on finding molecular and ADMET (absorption, distribution, metabolism, excretion and toxicity) properties and bioavailability scores of allicin for its use as a target specified drug. Past researches revealed that mercuric chloride, gold and silver inhibits AQP1 but due to their toxic effects *in vivo* they setback their application in treatment for brain cancer [24]. This challenge can be copped by focusing on natural compounds with high blood brain barrier permeability [25] (**Figure 1**).

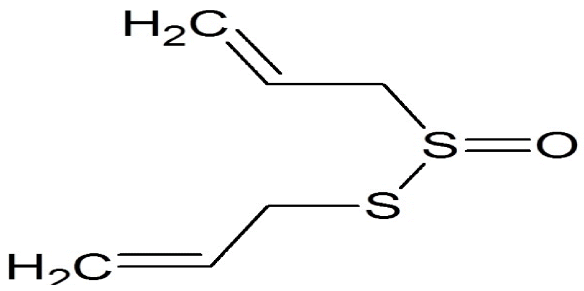

Figure 1: Structure of Allicin.

Methods

Molecular docking

The chemical structure of allicin was made by ACD Chem Sketch software (I). Molecular docking of allicin with AQP1, AQP4, AQP5 and AQP9 were performed by using Autodock4. The 3D structure of AQP1, AQP4, AQP5 and AQP9 (PDB ID 1H6I, 2D57, 5C5X and 1LDF) were acquired from RCSB (Research Collaboratory for Structural Bioinformatics). The 3D structure of AQP9 used for this work was of E. coli glycerol facilitator transport protein which has 43% sequence identity with that of human AQP9 according to homology modelling results. The PDB 3D coordinate file for allicin was generated by online smile translator by using smile notation from PubChem.

Hetero atoms along with water molecules were removed from pdb files of AQPs with the help of Autodock and all non-polar H-atoms were merged. Adaptive local search based Lamarkian algorithm was applied for search parameter. Electrostatic interactions, hydrogen bonding, short range Van der Waals forces and entropy losses were considered as scoring function of autodock which was energy based.

Kollman charges for AQP1, AQP4, AQP5 and AQP9 of -94.914, -86.244, -743.649 and -95.825 respectively were added. The Lamarkian genetic algorithm parameters applied in this evaluation were: no. of runs=30, max no. of evaluations=2500000, population size=150, rate for mutations in gene=0.02, no. of generations=27000 and crossing over rate=0.8. Blind docking was performed using the grid of size 126,126 and 126 along X, Y, Z axis using spacing of 0.375 Å. The grid centres were set at x=2.788, y=69.724 and z=4.814 for AQP1; x=18.35, y=42.248 and z=37.507 for AQP4; x=30.21, y=48.151 and z=7.963 for AQP5 and x=30.184, y=59.609 and z=157.413 for AQP9. The RMS tolerance of cluster was set at 2 Å.

ADMET, molecular and bioavailability scores

The ADME (absorption, distribution, metabolism and excretion) properties were calculated by using smile notation in Swiss ADME web based tool [26]. The toxicity was calculated by using PROTOX web based tool [27]. The molecular properties and bioavailability scores were calculated by using Molinspiration web based tool.

Results

Molecular docking

In this study molecular docking was performed to analyse structural inhibition of allicin against AQPs. The outcomes of our molecular docking of AQPs with allicin gave significant binding energies of -4.76 kcal/mol for AQP1, -4.89 kcal/mol for AQP4, -4.19 kcal/mol for AQP5 and -4.78 kcal/mol for AQP9, with 1H-bond in each docked conformation proving effective bindings between the allicin and AQPs.

The docking interactions are shown in **Figure 2** and compiled results are shown in **Table 1**.

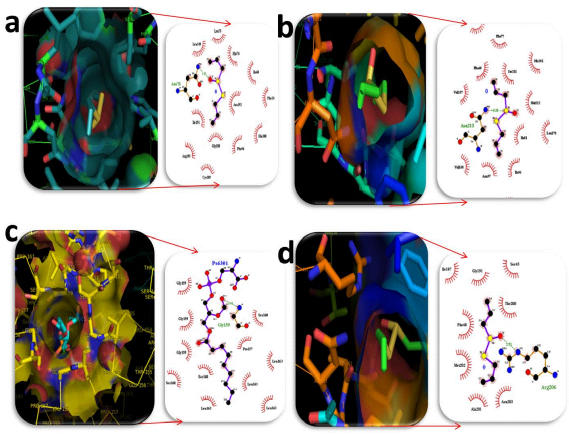

Figure 2: Docking interaction of Allicin with: a) AQP1 forming one H-bond at ASN76, b) AQP4 forming one H-bond at ASN213, c) AQP5 forming one H-bond at GLY159, and d) AQP9 forming one H-bond at ARG206.

Table 1: Allicin docked with brain aquaporins.

Allicin docked with	Binding energy(kcal/mol)	No. of H-bonds	Van der waal Energy(kcal/mol)	Residues
AQP1	-4.76	1	-6.17	ASN76
AQP4	-4.89	1	-6.41	ASN213
AQP5	-4.19	1	-5.61	GLY159
AQP9	-4.78	1	-6.24	ARG206

ADMET, molecular and bioavailability scores

The ADME properties, t he m olecular properties an d the bioavailability scores of allicin are given in **Tables 2-9**.

Toxicity analysis of allicin assigned it to toxicity Class IV with LD50 (Lethal dose 50 is the intake amount of a substance at which is capable of killing 50% test samples) of 874 mg/kg.

Table 2: Physiochemical properties of allicin.

Formula	$C_6H_{10}OS_2$
Molecular weight	162.27 g/mol
Num. heavy atoms	9
Num. arom. heavy atoms	0
Fraction Csp3	0.33
Num. rotatable bonds	5
Num. H-bond acceptors	1
Num. H-bond donors	0
Molar Refractivity	45.88
TPSA	61.58 $Å^2$

Table 3: Lipophilicity of allicin.

Log $P_{o/w}$ (iLOGP)	1.95
Log $P_{o/w}$ (XLOGP3)	1.31
Log $P_{o/w}$ (WLOGP)	2.62
Log $P_{o/w}$ (MLOGP)	1.18
Log $P_{o/w}$ (SILICOS-IT)	0.96
Consensus Log $P_{o/w}$	1.61

Table 4: Water solubility of allicin.

Log S (ESOL)	-1.34
Solubility	7.39e+00 mg/ml; 4.56e-02 mol/l
Class	Very soluble
Log S (Ali)	-2.2
Solubility	1.02e+00 mg/ml; 6.26e-03 mol/l
Class	Soluble
Log S (SILICOS-IT)	-1.7
Solubility	3.24e+00 mg/ml; 2.00e-02 mol/l
Class	Soluble

Table 5: Pharmacokinetics of allicin.

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log Kp (skin permeation)	-6.36 cm/s

Table 6: Drug likeness of allicin.

Lipinski	Yes; 0 violation
Ghose	No; 1 violation: #atoms<20
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55

Table 7: Medicinal chemistry of allicin.

Pains	0 alert
Brenk	2 alerts: disulphide, isolated_alkene
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	3.6

Table 8: Molecular properties.

miLogP	2.06
TPSA	17.07
Natoms	9
MW	162.28
nON	1
Nohnh	0
Nviolations	0
Nrotb	5
Volume	145.51

Table 9: Bioavailability scores.

GPCR ligand	-2.51
Ion channel modulator	-2.26
Kinase inhibitor	-2.95
Nuclear receptor ligand	-2.66
Protease inhibitor	-1.4
Enzyme inhibitor	-1.52

This shows that it is least harmful if taken in amount below 874 mg/kg body weight. Also no possible toxic target binding was found proving it safe for use as oral drug.

Discussion

Brain cancers being the most prevalent and fatal one out of all cancer types are extending their arms to engulf a huge amount of population [1]. Researches are going on to find effective treatments but due to the presence of blood brain barrier, the treatments are facing hindrance proving that brain cancers are not easy to reach by present treatments. Many treatments pose side effects due to undesired and wrongly targeted action of drug [14,24]. Also there is need to find

potent targets to develop efficient treatment for malignant conditions. In this study a well-established natural compound for cancer treatment, allicin is considered for analysing its inhibitory effect on AQPs which are the least studied targets in concerns with brain cancer. Our work revealed that structural inhibition of AQPs by allicin is an effective one, with binding energies of -4.76 kcal/mol, -4.89 kcal/mol, -4.19 kcal/mol and -4.78 kcal/mol for AQP1, AQP4, AQP5 and AQP9 respectively. There was 1hydrogen bond found in each docking interaction. Other studies reported tetra ethyl ammonium and acetazolamide as inhibitors of AQP1 but by analysing their ADMET properties using Swiss ADME and PROTOX we found that both of them have negative log P value showing least lipophilic nature thus they are not permeant to blood brain barrier also acetazolamide have affinity to bind possible toxic targets like amine oxidase A and prostaglandin G/H synthase 1 [24,26,27]. Another inhibitor reported for both AQP1 and AQP4 is arylsulphonamide AqB013, ADME properties analysis and molecular properties analysis by molinspiration, conclude that it is not permeant to blood brain barrier due to its very low Log P value and thus less lipophilic [25,26].

The water transporting property of AQP4 is likely to conclude that it has high affinity for water molecules and hence has tendency to form H-bonds *in vivo*. In a docking study of AQP4 with water, binding energy of -1.97 kcal/mol was reported which is less as compared to our result for allicin showing that our compound allicin with binding energy of -4.89 kcal/mol is having more efficient binding with AQP4 than water [13]. Till date, inhibitors of AQP5 and AQP9 are not yet well characterized and thus our study is an initial effort in this direction [16,20]. So, in light of our finding and above denoted facts we conclude that that allicin is an effective inhibitor of AQPs and thus can reduce edema, metastasis, angiogenesis and invasion of tumor cells in brain cancer to a great extent. In addition to effective inhibition of AQPs, according to our ADME analysis allicin follows Lipinski's rules with no violation and its log P value proves its ability to pass blood brain barrier [26]. Also its natural antioxidant and anti-inflammatory properties in turn contributes to reduce the adversity of malignant conditions [21]. Hence, allicin is an effective all-rounder to efficiently treat brain cancer and demands extensive research in this context.

Conclusion

Brain cancers are becoming more widespread in today's scenario and their diversity is increasing at high pace thus an effective and efficient treatment is an urgent need of present times. Due to the presence of blood brain barrier many drugs are incapable to reach the targets inside the brain and also pose unwanted toxic effects if diverted from its original target. So, our study of inhibition of AQPs with a natural compound allicin is an initial effort which overcomes the following drawbacks and is an effective and efficient treatment having promising future prospects and demands more extensive research to fight brain cancer.

Author's Contributions

Both the authors have equal contribution. All authors read and approved the final manuscript.

Competing Interests

The authors have declared no competing interests.

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