Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ • Экспериментальные и теоретические статьи •
• Experimental and theoretical articles •

Biolog. Journal of Armenia, 2 (71), 2019

ASSESSMENT OF THE THERAPEUTIC POTENTIAL OF ANTI-MALARIAL DRUGS AS *β*-AMYLOID INHIBITORS USING MOLECULAR MODELING METHODS

Y.R. HAMBARDZUMYAN[1], S.V. GINOSYAN[2], S.G. TIRATSUYAN[1]

[1]*Russian-Armenian University, Institute of Biomedicine and Pharmacy, Department of Bioengineering, Bioinformatics and Molecular Biology*
[2]*Russian-Armenian University, Institute of Biomedicine and Pharmacy, Department of Medical Biochemistry and Biotechnology*
siranush.ginosian@student.rau.am

The interaction of some secondary metabolites of the medicinal plant *Artemisia annua* with the amyloidogenic peptide 5A$\beta_{17\text{-}42}$ was studied *in silico*. Docking analysis of artemisinin, dihydroartemisinin and dimer of dihydroartemisinin with 5A$\beta_{17\text{-}42}$ was performed. The results were compared with curcumin, which is at stage II of clinical trials, and with the nonsteroidal anti-inflammatory drug ibuprofen. It is shown that all five ligands have a fairly high binding energy, with a maximum for dimer of dihydroartemisinin. All studied ligands interact directly with the most important sites of 5A$\beta_{17\text{-}42}$ peptide. Artemisinins as well as curcumin and ibuprofen, can suspend the formation and growth of 5A$\beta_{17\text{-}42}$ fibril, while dihydroartemisinin and dimer of dihydroartemisinin can destabilize already formed amyloid.

β-amyloid peptide – curcumin – ibuprofen – artemisinin – dihydroartemisinin – dimer of dihydroartemisinin

In silico հետազոտվել է *Artemisia annua* դեղաբույսի որոշ երկրորդային մետաբոլիտների փոխազդեցությունն ամիլոիդածին 5A$\beta_{17\text{-}42}$ պեպտիդի հետ: Իրականացվել է արտեմիզինինի, երկհիդրոարտեմիզինինի, երկհիդրոարտեմիզինինի դիմերի 5A$\beta_{17\text{-}42}$ ֆիբրիլի հետ դոքինգի վերլուծություն: Այն համեմատվել է կուրկումինի դոքինգի հետ, որը գտնվում է կլինիկական փորձարկումների 2-րդ փուլում, ինչպես նաև ոչ ստերոիդային հակաբորբոքային դեղամիջոց իբուպրոֆենի հետ: Ցույց է տրվել, որ բոլոր լիգանդները ունեն բավականաչափ բարձր կապման էներգիա, որն առավելագույն արժեքն ունի երկհիդրոարտեմիզինինի դիմերի դեպքում: Բոլոր լիգանդները անմիջականորեն փոխազդում են 5A$\beta_{17\text{-}42}$ պեպտիդի կարևորագույն կայքերի հետ: Արտեմիզինինները կուրկումինի և իբուպրոֆենի նման կարող են կանգնեցնել 5A$\beta_{17\text{-}42}$ ֆիբրիլի առաջացումը, իսկ երկհիդրոարտեմիզինինը և երկհիդրոարտեմիզինինի դիմերը կարող են նաև ապակայունացնել արդեն առաջացած ամիլոիդը:

β-ամիլոիդային պեպտիդ – կուրկումին – իբուպրոֆեն – արտեմիզինին – երկհիդրոարտեմիզինին – երկհիդրոարտեմիզինինի դիմեր

In silico исследовано взаимодействие некоторых вторичных метаболитов лекарственного растения *Artemisia annua* с амилоидогенным пептидом 5A$\beta_{17\text{-}42}$. Проведен докинг анализ артемизинина, дигидроартемизинина и димера дигидроартемизинина с 5A$\beta_{17\text{-}42}$, результаты которого сравнивали с докингом куркумина, который находится на II этапе клинических испытаний, и нестероидного противовоспалительного препарата ибупрофена.

Показано, что все пять лигандов имеют довольно высокую энергию связывания, с максимальной для димера дигидроартемизинина. Все исследуемые лиганды непосредственно взаимодействуют с важнейшими сайтами 5A$\beta_{17\text{-}42}$ пептида. Артемизинины аналогично куркумину и ибупрофену могут приостанавливать образование и рост фибриллы 5A$\beta_{17\text{-}42}$, а дигидроартемизинин и димер дигидроартемизинина могут дестабилизировать уже образовавшийся амилоид.

β-амилоидный пептид – куркумин – ибупрофен – артемизинин – дигидроартемизинин – димер дигидроартемизинина

Alzheimer's disease (AD) is the most common cognitive disorder among elderly population. It is characterized by deposition of *β*-amyloid (A*β*) peptides, neurofibrillary tangles, astrogliosis and microgliosis, which lead to neuronal dysfunction and neurodegeneration [1]. Numerous epidemiological studies confirm that long-term use of nonsteroidal anti-inflammatory drugs prevents AD, but does not affect the progression of the disease in already diagnosed patients [2]. It has been shown that some secondary metabolites of plants, including artemisinins, demonstrate antioxidant, anti-inflammatory, anti-amyloidogenic, neuroprotective and cognitive-stimulating features [14].

One of the main hallmarks of Alzheimer's disease is the formation of protein plaques, which consist of amyloid peptides of various lengths in the brain. Accumulation of aggregated *β*-amyloid peptides in the brain plays a key role in the neuropathology of Alzheimer's disease and in the neurotoxicity associated with it. The mechanism of peptide aggregation is an object of interest, which is intensely debated nowadays. There are several strategies used for AD treatment. One of them is based on prevention of peptide generation by suppressing the activity of *β*- and *γ*-secretases that promote the formation of A*β* peptides. Another strategy is based on the suppression of misfolding and on reversible aggregation [15]. A large number of different origin inhibitors of amyloidogenic peptide aggregation was found. Nevertheless, there is no effective treatment for AD, which makes the development of new inhibitors based on the amyloid cascade hypothesis very relevant [8]. One of the modern approaches dedicated to the search of potential drugs is the investigation of the interactions of metabolites mentioned above with *β*-amyloid using molecular modeling [13].

One of the main problems in the development of drugs for the AD treatment is that the sites of small molecules in 5A$\beta_{17\text{-}42}$ are not known *a priori*. Therefore, molecular docking is used to determine the possible binding sites location.

In the present study we investigated the nature of the interaction of artemisinin (ART), dihydroartemisinin (DHA) and dimer of dihydroartemisinin (DDHA) with 5A$\beta_{17\text{-}42}$ peptide using docking analysis and compared with curcumin (CUR), which is at stage II of clinical trials and nonsteroidal anti-inflammatory drug ibuprofen (IBU).

Materials and methods. 3D structures of CUR [CID: 969516], IBU [CID: 3672], ART [CID: 68827], DHA [CID: 456410] and DDHA [CID: 44564070] were obtained from PubChem database [4]. Ligand topologies were generated using acpype [6]. The 3D structure of 5A$\beta_{17\text{-}42}$ fibril in PDB format was taken from the RCSB Protein Data Bank database (PDB ID: 2BEG) [3]. Docking analysis was performed using Autodock Tools and Autodock Vina [16] at the exhaustiveness equal to 512 with the grid box values describing the entire surface of the fibril. The Ligplot^{+} program was used to analyze hydrophobic interactions and hydrogen bonds [17]. The data visualization was performed by PyMOL [7]. Permeability across the blood-brain barrier (BBB) and human intestinal absorption (HIA) was calculated using the PreADME software [5]. The ability to intersect via BBB is measured [12].

Results and Discussion. The 3D structure of 5A$\beta_{17\text{-}42}$ fibril contains ten models obtained by NMR [11]. We carried out a docking analysis for five ligands with 10 given models. The 8th model of 5A$\beta_{17\text{-}42}$ demonstrated the highest average binding energy for all five ligands (tab. 1). Further studies were performed on this model.

Table 1. The average values of binding energies from the results of the docking analysis for ligands with ten models of the peptide 5A$\beta_{17\text{-}42}$

Models	1	2	3	4	5	6	7	8	9	10
	ΔG_b (kcal/mol)									
CUR	-6,4 ±0.3	-5,1 ±0,18	-6,1 ±0,18	-5,9 ±0,18	-5,5 ±0,14	-6,9 ±0,3	-5,5 ±0,31	-7,6 ±0,44	-5,7 ±0,19	-6,5 ±0,39
IBU	-5,3 ±0,5	-4,9 ±0,3	-4,97 ±0,26	-4,98 ±0,35	-4,96 ±0,47	-6,4 ±0,55	-4,9 ±0,26	-6,7 ±0,52	-5,08 ±0,38	-5,4 ±0,32
ART	-5,5 ±0,3	-5,5 ±0,15	-5,8 ±0,3	-5,5 ±0,18	-5,6 ±0,2	-6,04 ±0,4	-5,4 ±0,25	-6,3 ±0,41	-5,5 ±0,15	-5,8 ±0,29
DHA	-5,4 ±0,28	-5,4 ±0,25	-5,7 ±0,2	-5,3 ±0,21	-5,4 ±0,22	-5,9 ±0,38	-5,4 ±0,32	-6,1 ±0,43	-5,4 ±0,17	-5,8 ±0,24
DDHA	-6,5 ±0,5	-7,05 ±0,52	-6,9 ±0,66	-7,2 ±0,33	-7,09 ±0,44	-7,09 ±0,29	-6,6 ±0,24	-7,9 ±0,45	-7,07 ±0,26	-7,2 ±0,29

According to our results CUR and IBU have the same binding site between the β_1 and β_2 turns of the 5A$\beta_{17\text{-}42}$ peptide, which is consistent with literature data (fig. 1) [10, 2].

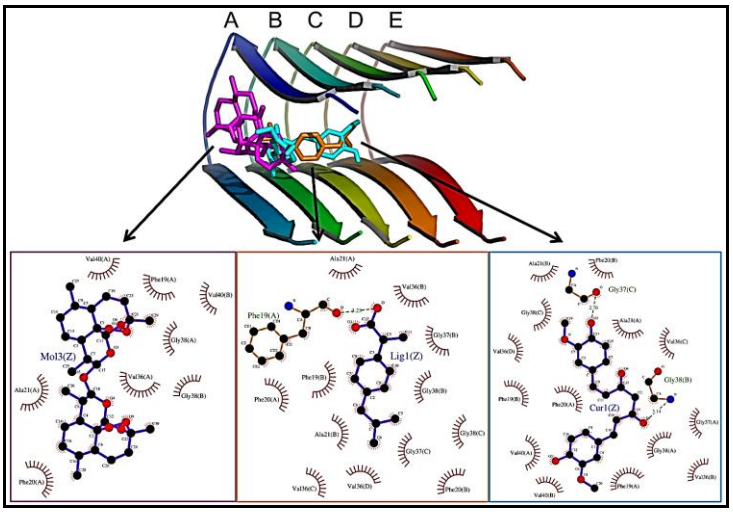

Fig. 1. Docking and analysis of hydrophobic interactions and hydrogen bonds of 1st site of DDHA (magenta), IBU (orange) and CUR (cyan) with 5A$\beta_{17\text{-}42}$ fibril.

Curcumin forms 2 hydrogen bonds with the Gly38 of chain B, Gly37 of chain C and many hydrophobic interactions with a binding energy of 8.4 kcal/mol. Ibuprofen forms a hydrogen bond with the Phe19 of chain A and many hydrophobic interactions with a binding energy of -7.7 kcal/mol. The dimer of dihydroartemisinin has 2 binding sites, the first one coinciding with the CUR's binding site (fig. 1). The binding energy of DDHA (-8.3 kcal/mol) practically equals with binding energy of CUR. The dimer of DHA forms hydrogen bonds and hydrophobically interacts with almost the same amino acids as CUR. However, DDHA positioning is different from IBU and CUR, which interact with the amino acid residues of chains A, B, C and D at the same time DDHA interacts with the amino acids of chains A and B.

In the second site, DDHA forms a hydrogen bond with Asn27 (C) (fig. 2). The binding energy is -8.0 kcal/mol. In this site the DDHA hydrophobically interacts with amino acid residues of the B, C, D, E chains, such as Asp27 (B), Lys28 (C), Gly29 (C), Asp27 (D), Lys28 (D), Ala30 (D), Asp27 (E), Gly29 (E). It should be noted that Lys28 of C and D chains are involved in stabilization of the 5A$\beta_{17\text{-}42}$ fibril [10].

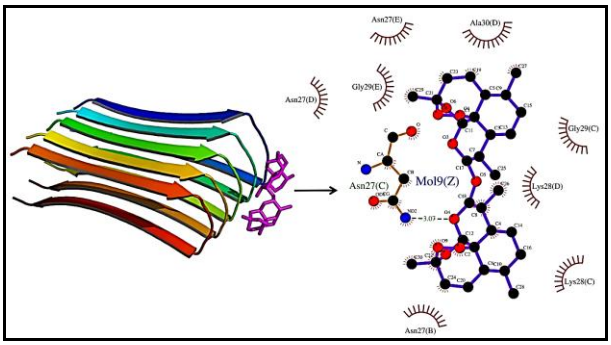

Fig. 2. Docking and analysis of hydrophobic interactions and hydrogen bonds of 2nd site of DDHA (magenta) with 5A$\beta_{17\text{-}42}$ fibril

Artemisinin interacts with the site of 5A$\beta_{17\text{-}42}$ formed by the N- and C-terminal of chains C, D and E with a binding energy of -7.2 kcal/mol without forming hydrogen bonds (fig. 3). It shows hydrophobic interactions with Leu17 (C), Phe19 (C), Val18 (D), Phe19 (D), Leu17 (E), Val18 (E), Phe19 (E), Val39 (E) and Val40 (E).

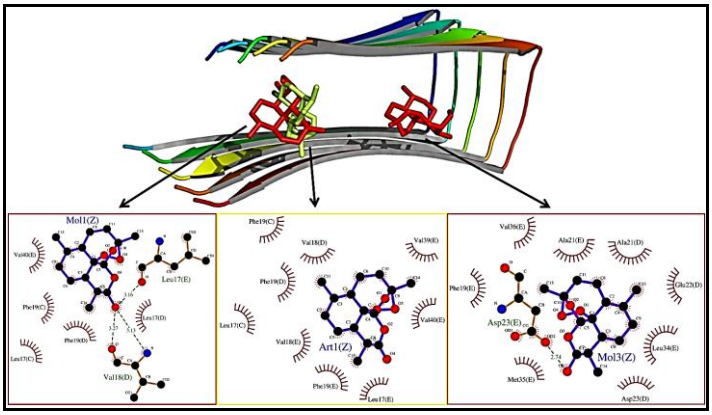

Fig. 3. Docking and analysis of hydrophobic interactions and hydrogen bonds of DHA (red) and ART (yellow) with 5A$\beta_{17\text{-}42}$ fibril.

Dihydroartemisinin shows two binding sites with 5A$\beta_{17\text{-}42}$. The first coincides with the ART's binding site with a binding energy of - 6.9 kcal/mol. At this site, DHA forms 2 hydrogen bonds with the Val18 of chain D and the Leu17 of chain E with many hydrophobic interactions. In the second site, DHA forms a hydrogen bond with the Asp23 (E), which is involved in stabilization of 5A$\beta_{17\text{-}42}$ fibril (ΔG_b = -6.8 kcal/mol) (fig. 3).

The analysis shows that in some cases, for example, the binding of CUR, IBU, DHA with 5A$\beta_{17\text{-}42}$ the hydrogen bond plays an important role. However, for ART and DDHA the hydrogen bonds do not play a role depending on the binding site of 5A$\beta_{17\text{-}42}$. At the same time, DDHA forms one hydrogen bond in the 2nd site. This situation occurs

when the number of HB donors and ligand acceptors is small (ART has only one HB donor and one HB acceptor). In this case, the electrostatic and van der Waals interactions become dominant.

Pharmacological characteristics, such as HIA and BBB were analyzed for the studied compounds (tab. 2). It is known that compounds with log(BB) > 0.3 can cross the BBB easily, unlike ones with log(BB) < -1.0 [5]. It can be concluded that all ligands are able to overcome the BBB to one degree or another. At the same time they have a very high absorbability.

Table 2. Physico-chemical properties and binding energies for CUR, IBU, ART, DHA and DDHA

CID	Compound	ΔG_b (kcal/mol)	Log(BB)	HIA (%)
969516	CUR	-8.4	-1.039	94.40
3672	IBU	-7.7	0.103	98.38
68827	ART	-7.2	0.116	96.31
456410	DHA	*-6.9 **-6.8	-0.003	93.58
44564070	DDHA	*-8.3 **-8.0	-0.648	99.06

*-1st site
**-2nd site

Tab. 2 shows that the studied artemisinins have high BBB permeability values, which indicates their high bioavailability. They can be used as therapeutic agents for the treatment of CNS diseases as reported in the literature [14]. It has been noted that there is no information regarding to the dimer of dihydroartemisinin.

It is known that CUR, IBU and ART indirectly interfere with the aggregation of Aβ [18, 14]. According to our results they also directly bind to 5A$\beta_{17\text{-}42}$ fibril. Hence, we can conclude that inhibition of the formation of fibrils is mediated by a direct binding mechanism that modulates the ability to aggregate. It is known that the formation and further growth of fibrils occur due to amino acid residues Leu17-Ala21 and Gly37-Ala42, located in β_1 and β_2, respectively [9]. Thus, for the first time it has been shown that ART, DHA and DDHA can suspend the formation and growth of 5A$\beta_{17\text{-}42}$ fibril similarly to CUR and IBU. The already formed fibril is stabilized at the expense of Lys28 and Asp23 in the turn region between β_1 and β_2. These amino acids form a salt bridge with a bound layer of water [10]. This means that DHA and DDHA can destabilize the already formed Aβ_{42}.

Acknowledgement

We are grateful for the financial support to the Ministry of Education and Science of the Republic of Armenia (grant № 10-2/I-1).

REFERENCES

1. *Alzheimer's Association.* Alzheimer's disease facts and figures. Alzheimers Dement, *14*, 3, 367-429, 2018.
2. *Azam F., Alabdullah N.H., Ehmedat H.M., Abulifa A.R., Taban I., Upadhyayula S.* NSAIDs as potential treatment option for preventing amyloid β toxicity in Alzheimer's disease: an investigation by docking, molecular dynamics, and DFT studies. J. Biomol. Struct. Dyn., *36*, 8, 2099-2117, 2018.

3. *Berman H.M., Westbrook J., Feng Z., Gilliland G., Bhat T.N., Weissig H., Shindyalov I.N., Bourne P.E.* The protein data bank. Nucleic Acids Res., *28*, 1, 235-242, 2000.
4. *Bolton E.E., Wang Y., Thiessen P.A., Bryant S.H.* PubChem: integrated platform of small molecules and biological activities. Annu Rep. Comput. Chem., Elsevier, *4*, 217-241, 2008.
5. *Clark D.E.* Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood–brain barrier penetration. J. Pharm. Sci., *88*, 8, 815-821, 1999.
6. *da Silva A.W.S., Vranken W.F.* ACPYPE-Antechamber python parser interface. BMC Res. Notes, *5*, 1, 367, 2012.
7. *DeLano W.L.* Pymol: An open-source molecular graphics tool. CCP4 Newsletter On Protein Crystallography, *40*, 82-92, 2002.
8. *Demuro A., Smith M., Parker I.* Single-channel Ca^{2+} imaging implicates A*β*1–42 amyloid pores in Alzheimer's disease pathology. J. Cell Biol., *195*, 3, 515-524, 2011.
9. *Han W., Schulten K.* Fibril elongation by Aβ17–42: Kinetic network analysis of hybrid-resolution molecular dynamics simulations. J. Am. Chem. Soc., *136*, 35, 12450-12460, 2014.
10. *Kundaikar H.S., Degani M.S.* Insights into the Interaction mechanism of ligands with A*β*42 based on molecular dynamics simulations and mechanics: implications of role of common binding site in drug design for alzheimer's disease. Chem. Biol. Drug Des., *86*, 4, 805-812, 2015.
11. *Lührs T., Ritter C., Adrian M., Riek-Loher D., Bohrmann B., Döbeli H., Schubert D., Riek R.* 3D structure of Alzheimer's amyloid-*β* (1-42) fibrils. PNAS, *102*, 48, 17342-17347, 2005.
12. *Ngo S.T.* Interactions between small molecules and amyloid beta peptides: Implications for Alzheimer's disease (Doctor of Philosophy thesis, Institute of Physics Polish Academy of Sciences), 2015.
13. *Okorji U.P.* Inhibition of neuroinflammation by artemisinin and its derivatives (Doctoral dissertation, University of Huddersfield), 2015.
14. *Shi Z., Chen Y., Lu C., Dong L.M., Lv J.W., Tuo Q.H., Qin L., Cheng S.W., Bu L.L., Lin N., Zhu X.X., Liao D.F., Liu X.M.* Resolving neuroinflammation, the therapeutic potential of the anti-malaria drug family of artemisinin. Pharmacol. Res., *136*, 172-180, 2018.
15. *Sun X., Jin L., Ling P.* Review of drugs for Alzheimer's disease. Drug Discov. Ther., *6*, 6, 285-290, 2012.
16. *Trott O., Olson A.J.* AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem., *31*, 2, 455-461, 2010.
17. *Wallace A.C., Laskowski R.A., Thornton J.M.* LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. Des. Sel., *8*, 2, 127-134, 1995.
18. *Yang F., Lim G.P., Begum A.N., Ubeda O.J., Simmons M.R., Ambegaokar S.S.,Chen P.P., Kayed R., Glabe C.G., Frautschy S.A., Cole G.M.* Curcumin inhibits formation of amyloid *β* oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. J. Biol. Chem., *280*, 7, 5892-5901, 2005.

Received on 08.04.2019