25. *Roth, D. G. and T. F. Deuel* (1974). "Stability and regulation of phosphoribosyl pyrophosphate synthetase from rat liver." J. Biol. Chem.249: 297-301.
26. *Roth, D. G., E. Shelton and T. F. Deuel* (1974). "Purification and properties of phosphoribosyl pyrophosphate synthetase from rat liver." J. Biol. Chem.249: 291-296.
27. *Switzer, R. L.* (1969). "Regulation and mechanism of phosphoribosyl pyrophosphate synthetase. I. Purification and properties of the enzyme from Salmonella typhimurium." J. Biol. Chem.244: 2854-2863.
28. *Switzer, R. L. and D. C. Sogin* (1973). "Regulation and mechanism of phosphoribosyl pyrophosphate synthetase. V. Inhibition by end products and regulation by adenosine diphosphate." J. Biol. Chem.248: 1063-1073.
29. *Valencia, A. and J. Morán* (2004). "Reactive oxygen species induce different cell death mechanisms in cultured neurons." Free Radic Biol Med36(9): 1112-1125.
30. *Widmer, R., M. Engels, P. Voss and T. Grune* (2007). "Postanoxic damage of microglial cells is mediated by xanthine oxidase and cyclooxygenase." Free Radic Res41(2): 145-152.
31. *Willemoes, M. and B. Hove-Jensen* (1997). "Binding of divalent magnesium by Escherichia coli phosphoribosyl diphosphate synthetase." Biochemistry36: 5078-5083.

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INSIGHTS ON GLUCOCORTICOID RECEPTOR MODULATION THROUGH BINDING OF ARTEMISININ

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In this work *in silico* study of the interaction of artemisinin with ligand bind domain of glucocorticoid receptor by molecular modeling methods. Artemisinins belong to the family of sesquiterpene lactones, secondary metabolites of medicinal plant Artemisia annua, which has been traditionally used in Chinese medicine. Artemisinins exhibit antioxidant, anti-inflammatory, anti-carcinogenic and other activities. Molecular docking, principal component analysis, cluster analysis have revealed three binding sites of artemisinin with ligand bind domain of glucocorticoid receptor which are very important regions. Comparative analysis was performed with dexamethasone, which is a corticosteroid medication. Thus, we have shown for the first time that artemisinin affects extremely important sites of ligand bind domain of glucocorticoid receptor, it should be noted that the first binding site of artemisinin corresponds to the interaction site of dexamethasone. This may represent a molecular basis for ligand-dependent receptor activation and the possibility of using artemisinin as a new ligand for glucocorticoid receptor.

Glucorticoid receptor – artemisinin – dexamethasone - molecular docking – cluster analysis

В данной работе изучено insilico взаимодействие артемизинина с лиганд связывающим доменом глюкокортикоидного рецептора методами молекулярного моделирования. Артемизинины относятся к семейству сесквитерпеновых лактонов, вторичных метаболитов лекарственного растения Artemisiaannua, которое традиционно используется в китайской медицине. Артемизинины проявляют антиоксидантную, противовоспалительную, антиканцерогенную и др. активности. Методами молекулярного докинга, анализа по главным компонентам и кластерного анализа выявлено три сайта связывания артемизинина с лиганд связывающим доменом глюкокортикоидного рецептора, которые являются чрезвычайно важными участками. Проведен сравнительный анализ с кортикостероидным препаратом дексаметазоном. Нами впервые показано, что артемизинин влияет на чрезвычайно важные участки лиганд связывающим доменом глюкокортикоидного рецептора, при этом первый сайт связывания артемизинина соответствует сайту взаимодействия дексаметазона. Это может представлять молекуляр-

ную основу для лиганд-зависимой активации рецептора и рассмотрения возможности использования артемизинина в качестве нового лиганда для этого рецептора.

Глюкокортикоидный рецептор – артемизинин – дексаметазон – молекулярный докинг – кластерный анализ

Այս աշխատանքում իրականացված է արտեմիզինինի և գլյուկոկորտիկոիդային ընկալիչի լիգանդ կապող դոմենի փոխազդեցության ուսումնասիրություն *insilico* մոլեկուլային մոդելավորման մեթոդների միջոցով: Արտեմիզինինները պատկանում են սեսքվիթերպենային լակտոնների ընտանիքին, հանդիսանալով Artemisiaannua դեղաբույսերի երկրորդային մետաբոլիտ, որն ավանդաբար օգտագործվում է չինական բժշկության մեջ: Արտեմիզինինները ցուցաբերում են հակաօքսիդանտային, հակաբորբոքային, հակաքաղցկեղածին և այլ ակտիվություններ: Մոլեկուլային դոքինգի, հիմնական բաղադրիչերի վերլուծության, կլաստերային վերլուծության միջոցով հայտնաբերվել է արտեմիզինինի և գլյուկոկորտիկոիդային ընկալիչի լիգանդկապող դոմենի միջ և կապման երեք կայք, որոնց չափազանց կարևւոր են: Կատարվել է համեմատական վերլուծություն դեքսամետազոնի որպես կորտիկոսթերոիդային դեղանյութի հետ: Այսպիսով, առաջին անգամ ցույց է տրվել, որ արտեմիզինը փոխազդում է գլյուկոկորտիկոիդային ընկալիչիլի լիգանդկապող դոմենի հետ չափազանց կարևոր կայքերի հետ, ընդորում նրա առաջին կայքը համապատասխանում է դեքսամետազոնի փոհազդեցության կայքի հետ: Սա կարող է մոլեկուլային հիմք հանդիսանալ լիգանդից կախված ընկալիչի ակտիվացման և արտեմիզինի դիտարկման հնարավորությանը որպես նոր լիգանդ:

Գլյուկոկորտիկոիդային ընկալիչ – արտեմիզինին – դեքսամետազոն – մոլեկուլային դոքինգ–կլաստերային վերլուծություն

Artemisinins, secondary metabolites of medicinal plants Artemisia annua belong to the family of sesquiterpene trioxane lactones, traditionally used in Chinese medicine [1, 6]. Artemisinins exhibit a wide range of biological activities, such as antioxidant, anti-inflammatory, anti-carcinogenic, immunomodulating, antimicrobial, antiamiloidogenic anthelmintic, antiviral,etc. [8,19]. Artemisinins may contribute to formation of free radicals, modulate multiple signaling pathways, including TLR, Syk tyrosine kinase, phospholipase C, phosphatidyl inositol-3 kinase / protein kinase C cascade, mitogen-activated protein kinases, β-catenins [11, 8], and also the transcriptional factors STAT-1/3/5, NF-κB , Sp1 and Nrf2 / ARE, as well as NR3. In vitro studies in human hepatocytes revealed the activation of the constitutive androstan receptor (CAR, NR1I3) [5] the main mechanism of artemisinin mediated induction of CYP3A4, CYP2B6, and ABCB1 [18]. However, the exact mechanisms of action or molecular targets are not well studied. There is a major task for the identification of molecular targets for artemisinin. Preliminary *in silico* screening using TargetNet [23] showed that the optimal target for artemisinin is the ligand-binding domain of the glucocorticoid receptor (LBD GR). Ketosteroid receptors including human glucocorticoid (hGR) play a vital role in the maintenance of numerous functions such as metabolic and homeostatic regulation through the interaction with glucocorticoids [16, 20].Glucocorticoids are known to play a significant role in orchestrating cell-cell communication, which is necessary for the coordination of development, growth, metabolism, immunity and used extensively for inflammatory disorders [7, 14]. However, this is a double-edged sword approach with beneficial therapeutic actions alongside serious adverse effects [22].It should be noted that a lot of modern research has been focused on the development of novel compounds such as selective glucocorticoid receptor agonists or selective glucocorticoid receptor modulators , that can be used as anti-inflammatory drugs.

In silico study was performed for the identification of binding sites of artemisinin with human glucocorticoid receptor and comparative analysis with common used drug Dexamethasone (9-fluoro-11b,17,21-trihydroxy-16 α methylpregna-1,4-diene-3,20-dione).

Materials and methods

LBD of hGR was selected from the Brookhaven (RCSB) Protein Data Bank (PDB) [3]. In order to validate the accuracy of the obtained results, co-crystallized dexamethasone ligand was extracted from the LBD of hGR and performed blind docking.To build the hGr–

ligand complex, using Autodock Vina [21]. 100 docking runs were performed. Principal component (PC) [12] and cluster analysis using K-means algorithm [10] was performed on docking results. Cluster quality was assessed using Davies–Bouldin Index (DBI) ,Silhouette Score, Dunn Index and the pseudo-F statistic (pSF or Calinski Harabasz) [2]. The root-square-mean-deviation (RMSD) was calculated using MDTraj library [13]. The RMSD between the predicted conformation and the observed X-ray crystallographic conformation was then determined.Maps of hydrophobic and hydrophilic interactions for artemisinin and dexamethasone with hGR were also generated. Ligplot was used for the identification of interaction modes of artemisinin [23]. 3D visualization was done using Pymol [9].

Results and discussion

The docking procedure accuracy was assessed by examining how closely the centroid [13] pose extracted from 100 runs (binding conformation) by re-docking of co-crystallized ligand 9-fluoro-11b,17,21-trihydroxy-16a-methylpregna-1,4-diene-3,20-dione (dexamethasone) resembles the binding mode obtained by X-ray crystallography. The RMSD value between the two superimposed poses of dexamethasone co-crystallized ligand, bound to hGR (PDB ID: 4UDC) and its conformation obtained after re-docking was 0.5 Å (Fig.1). The RMSD value for the predicted pose is less than 2.0 Å, which indicates that the prediction is of good quality [21].

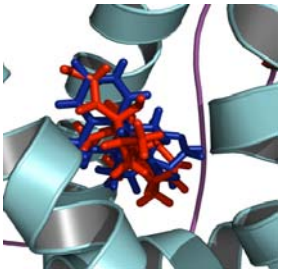
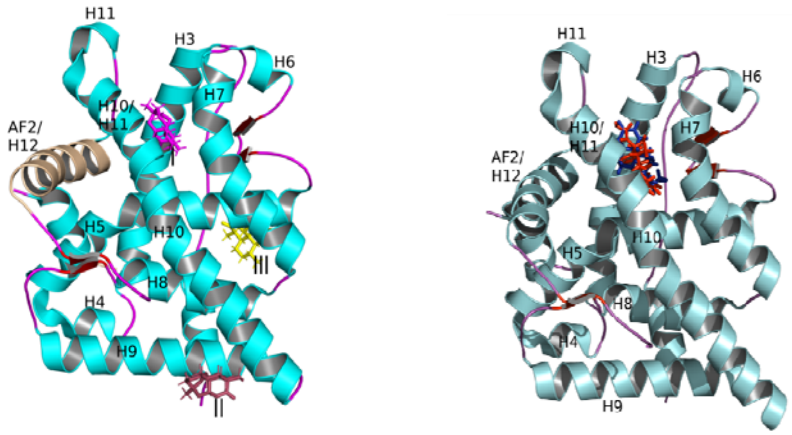


Figure 1. Superimposition of docked poses of dexamethasone (blue) with its crystallographic structure (red) conformation (RMSD: 0.5 Å).

Principal component analysis and cluster analysis using k-means demonstrated that there are three binding sites of artemisinin to LBD GR. The first binding site (cluster) contained 65% of docked poses with the highest binding affinity (-7,069±0,479kcal/mol). An optimal number of clusters were chosen, simultaneously accounting for a low DBI, High Silhouette, High Dunn Index and high pSF values [2].

Comparative analysis of the interaction of artemisinin and dexamethasone demonstrated that the first binding site of artemisinin matches the interaction site of dexamethasone with LBD hGR. (Fig 2 a, b).

Dexamethasone forms 3 hydrogen bonds with hGR between (Figure 3, Figure 4b) Leu 563, Gln 570 and Thr 739 residues. It should be noted that dexamethasone forms hydrogen bonds, while artemisin does not. Artemisinin interacts hydrophobically with Asn564, Gln642 и Tyr735 (Figure 3)



a b

Figure 2.a) Binding mode of artemisinin in the catalytic pocket of hGR (PDB ID: 4UDC): I, II and III – binding sites .b) Superimposition of docked pose of Dexamethazone with its crystallographic structure (RMSD: 0.5 Å)

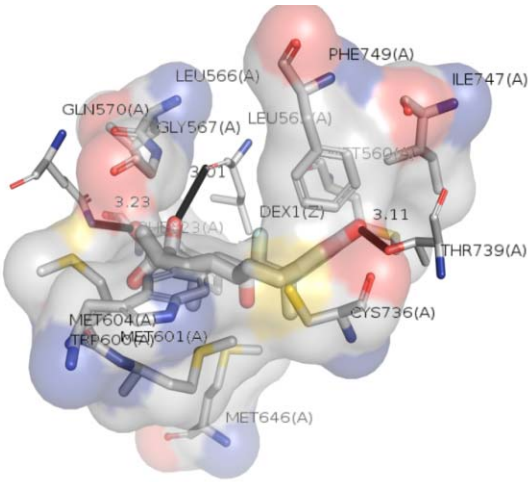
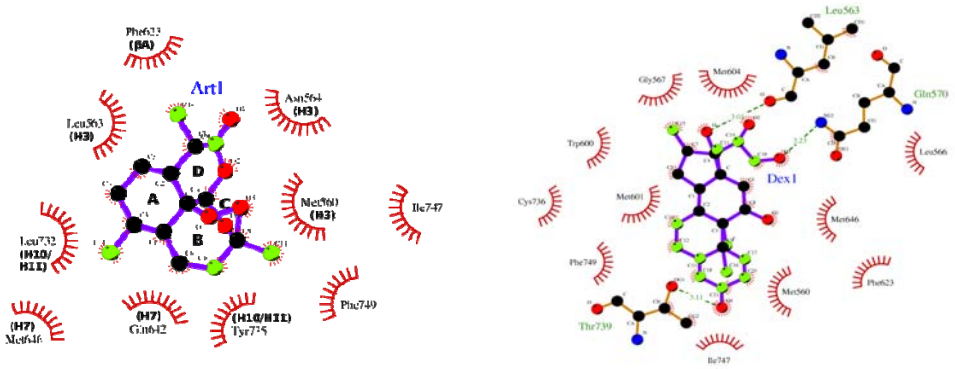


Figure 3. Putative Hydrogen bonds (black line) formed between Dexamethasone with LBD of hGR (PDB ID: 4UDC).

The hydrophobic interaction analysis of the first binding site demonstrated (fig 2a) that A, B, C и D rings of Artemisisin interact with α – helixes H3, H7, H10/H11 and βA-sheets of LBD GR. Aminoacids of H3 and βA play an important role during dimerization of LBD GR and aminoacids of H5 - dimer stabilization [4]. Aminoacids of H3-H7 (550-653a.a) are responsible for the binding with chaperone Hsp90[15], and H3, H5 и H12 - co-activator NcoA-2 binding site [23]. In the second binding site A,C,D rings of artemisinin interact hydrophobically with the aminoacids of H9 and H10 and in the third – H1,H5 and H8 with A,B,D rings. Aminoacids of Glu542, Val543(H1, H5) are responsible for the dimerization stabilization of LBD hGR[17] Artemisin interaction interacts hydrophobically with the aminoacids of the first binding site: Met560, Leu563, Phe623, Met646, Leu732, Ile747, Phe749, Arg611, 3rd binding site like dexamethasone.

All glucocorticoids interact with ligands including dexamethasone through Gln642, while Tyr735 plays an important role during ligand interpretation and receptor transactivation. The interactions with these amino acids play a critical role for conformation changes of the receptor. Both artemisinin and dexamethasone interact with Ile747 and Phe749 of the loop that is located before AF- 2 helix. These interactions provide the stabilization of AF-2 during its active state as well as the dimerization of LBD GR. This presents a molecular basis for ligand dependent activation of GR [4]. Comparative analysis of the first binding site of artemisinin with LBD GR with dexamethasone demonstrated that have common amino acids that participate during the interaction. Each ligand has its own peculiar interaction with other amino acids, which can explain GR conformation changes and this can promote the formation of alternative protein surface and affect co-regulator binding.



A B

Figure 4. Binding mode of the 1-st cluster artemisinin (PDB ID: 4UDC) (a) and dexamethasone (b) in the catalytic pocket of hGR.

In this study we have shown for the first time that artemisinin affects extremely important sites of LBD GR, it should be noted that the first binding site of artemisinin corresponds to the interaction site of dexamethasone. This may represent a molecular basis for ligand-dependent receptor activation and the possibility of using artemisinin as a new ligand for GR.

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References

1. *Коновалов Д. А., Хамилонов А. А.* Биологически активные соединения полыни однолетней. Эфирное масло. Фармация и фармакология, 2016.
2. *Сивоголовко Е. В.* Методы оценки качества четкой кластеризации. Компьютерные инструменты в образовании. 4, 96с, 2011.
3. *Berman, H., Westbrook, J., Feng, Z., et al.* The protein data bank. Nucleic acids research, 28,1, 235-242, 2000.
4. *Bledsoe R., Montana V., Stanley T.,* etc., Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell.110.1. p. 93-105. 2002.
5. *Burk O, Piedade R., Ghebreghiorghis L., et al.,* Differential effects of clinically used derivatives and metabolites of artemisinin in the activation of constitutive androstane receptor isoforms British Journal of Pharmacology.167, 666–681, 2012.

6. *Chougouo R. et al.* Anti-inflammatory and acetylcholinesterase activity of extract, fractions and five compounds isolated from the leaves and twigs of Artemisia annua growing in Cameroon. Springer Plus. 5,1 c, 1525, 2016.
7. *Cruz-Topete D. & Cidlowski J.* One Hormone Two Actions: Anti- and Pro-inflammatory Effects of Glucocorticoids, Neuroimmunomodulation. 22,0,20–32, 2015.
8. *Dai Y., Zhou W., Meng J., etc*. The pharmacological activities and mechanisms of artemisinin and its derivatives: a systematic review . Medicinal Chemistry Research. 26, 5, p. 867-880, 2017.
9. *DeLano W. L*. The PyMOL Molecular Graphics System. De-Lano Scientific, San Carlos, CA, USA .http:.www. pymol.org, 2002.
10. *Hartigan, J., & Wong M.* "Algorithm AS 136: A k-means clustering algorithm." Journal of the Royal Statistical Society. Series C (Applied Statistics) 28, 1, 100-108, 1979.
11. *Ho et al.* Artemisinins: pharmacological actions beyond anti-malarial. Pharmacology & therapeutics.142,1, c.126-139, 2014.
12. *Jolliffe, I*. Principal component analysis. John Wiley & Sons, Ltd, 2002.
13. *McGibbon, R., Beauchamp, K., Harrigan M., et al.* MDTraj: A modern open library for the analysis of molecular dynamics trajectories. Biophys. Journal.109, 8,1528-1532, 2015.
14. *Presman D., Hager G.* More than meets the dimer: What is the quaternary structure of the glucocorticoid receptor? .Transcription. 8,1, c 32-39, 2017.
15. *Ratajczak T., Allan R., Cluning C., & Ward B*. Functional Protein Interactions in Steroid Receptor-Chaperone Complexes. Protein Interactions. – InTech, 2012.
16. *Ratman D, Mylka V, Bougarne N, etc*. Chromatin recruitment of activated AMPK drives fasting response genes co-controlled by GR and PPAR_ Nucleic Acids Research. 44, 22, 10539–10553, 2016.
17. *Reyer H., Ponsuksili S., Kanitz E., et al.* A Natural Mutation in Helix 5 of the Ligand Binding Domain of Glucocorticoid Receptor Enhances Receptor-Ligand Interaction. PloS one. 11,10, e0164628, 2016
18. *Sangkuhl K,* Artemisinin and Derivatives Pathway, Pharmacokinetics, Clinical Pharmacology & Therapeutics. 92, 4, 414-417, 2012.
19. *Shi J., Zhang C., Sun X., et al.* Antimalarial Drug Artemisinin Extenuates Amyloidogenesis and Neuroinflammation in APPswe/PS1dE9 Transgenic Mice via Inhibition of Nuclear Factor-κB and NLRP3 Inflammasome Activation. CNS neuroscience & therapeutics. 19, 4, p. 262-268, 2013.
20. *Sundahl N., Bridelance J. et al.* Selective glucocorticoid receptor modulation: New directions with non-steroidal scaffolds .Pharmacology & therapeutics.152, p.28-41, 2015.
21. *Trott O., Olson A.* AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 31, 2, c. 455-461. 2010.
22. *Wallace A., Laskowski R., Thornton J.* LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein engineering. 8, 2, c.127-134, 1995.
23. *Weikum E, Matthew T. Knuesel, E. etc*., Glucocorticoid receptor control of transcription: precision and plasticity via allostery Nature Reviews Molecular Cell Biology. 18,159–174, 2017.
24. *Yao Z., Dong J., Che Y., et al*., TargetNet: a web service for predicting potential drug–target interaction profiling via multi-target SAR models. Journal of computer-aided molecular design. 30, 5, p. 413-424, 2016.
25. *Zhou W., Meng J., Du X., et al.* The pharmacological activities and mechanisms of artemisinin and its derivatives: a systematic review. Medicinal Chemistry Research. 26, 5, p. 867-880, 2017.