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EVALUATION OF ADEMOL MOLECULAR TARGET BY BIOINFORMATICS METHOD ACCORDING TO CRITERIA OF LIQUIDITY OF BIODACCESSIBILITY AND MOLECULAR DOCKING

Hodnotenie molekulovej štruktúry ademolu metódou bioinformatiky podľa kritérií biodaccessibility a molekulárneho dockingu

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SUMMARY
Using the method of bioinformatics, in particular, the method of molecular docking in silico on the cloned β1-adrenoceptor *Meleagris gallopavo*, the ability of ademol to block β-adrenoceptors was established. Depending on the cerebrospinal fluid activity and affinity for β1-adrenoceptors (Edoc, kkal/mol), ademol occupies an intermediate position between propranolol and timolol, and both of the latter reduce elevated intracranial pressure. The permeability of ademol through blood-brain barrier is a guarantee of its neuroprotective activity, and the structural similarity to β-blockers and its affinity determines the cerebrospinal fluid-potent action of the adamantane derivative, which causes a decrease in elevated intracranial pressure. Ademol is a promising neuroprotective agent that can be used in brain damage associated with high intracranial pressure (hemorrhagic stroke, traumatic brain injury).
Key words: *ademol, bioinformatics, intracranial pressure, stroke, traumatic brain injury, blood-brain barrier.*
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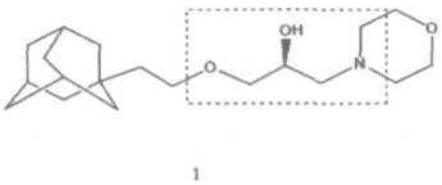
Introduction

The ability of ademol to correct elevated intracranial pressure (ICP) values can be explained by its specific structural and receptor features. The molecule of 1-adamantylethyloxy-3-morpholino-2-propanol hydrochloride (ademol) in its structure contains an adamantane nucleus similar to amantadine, and on the other hand has a structural similarity to the cardioselective β-blockers of propranolol and timolol (Fig. 1), pharmacophore, characteristic of all β-blockers with phenoxypropanolamine structure. Blockade of β-adrenoceptors of the vascular plexuses of the ventricles of the soft meninges leads to decrease ICP due to a decrease of cerebrospinal fluid production. We associate the presence of cerebrospinal fluid-hypotensive effect of ademol with β-adrenoblocking properties. The basis of this statement is supplemented by the fact that ademol has certain structural identities with propranolol (5, 8). Moreover, this action is realized not only due to the possible blockade of β-adrenoceptors, but also due to the activating effect of β-adrenoblockers on the functioning of 5-hydroxytryptamine receptors (5GT) of the venules of the sinuses of the meninges (3). This mechanism underlies the improvement of cerebrospinal fluid dynamics in the cerebrospinal fluid, resorption of cerebrospinal fluid, normalization of the balance between production and reverse absorption of cerebrospinal fluid and blood flow through the sinuses into the jugular veins, which reduces ICP. Given the above facts, it is appropriate to make the assumption that the cerebrospinal fluid antihypertensive effect of ademol, as in β-blockers, may also be associated with its effect on 5GT

Figure 1. Structural similarity of organic bases of ademol (1) and known β-blockers - timolol (2), D,L-propranolol (3), and selective β3-blocker SR 59230A (4) by propanamine pharmacophore (highlighted by dashes) **and their SMILE** (simplified molecular-input line-entry system) **- codes.**



1

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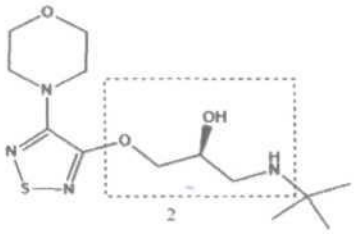

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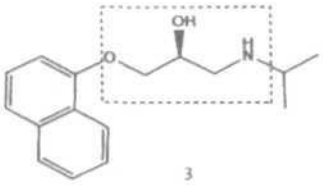

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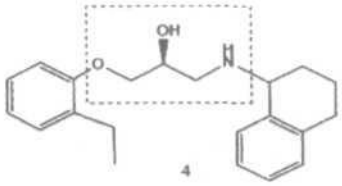


4

SMILES CCc1ccccc1OCC(CNC1CCCc2c1cccc2)O

receptors in the venules of the brain, which requires further research.

For a potential neuroprotector that exhibits cerebrospinal fluid hypotensive action, its sufficient permeability through the blood-brain barrier is mandatory, which is understandable and logical, as it ensures delivery of the drug directly to the target and is a guarantee of its binding to relevant receptors (NMDA, β-, or 5-GT receptors).

The aim of the work. The method of bioinformatics to justify the intravenous use of ademol, to identify the ability of ademol to block β-adrenoceptors, as well as the criteria of drug and bioavailability to assess the possibility of its passage through the blood-brain barrier compared with reference β-blockers (structural analogues of propanamine pharmacophore).

Materials and Methods

The research algorithm consisted of molecular modeling of the binding of a collection of compounds to beta-1-adrenoceptors *in silico* by binding energy (bioinformatics method).

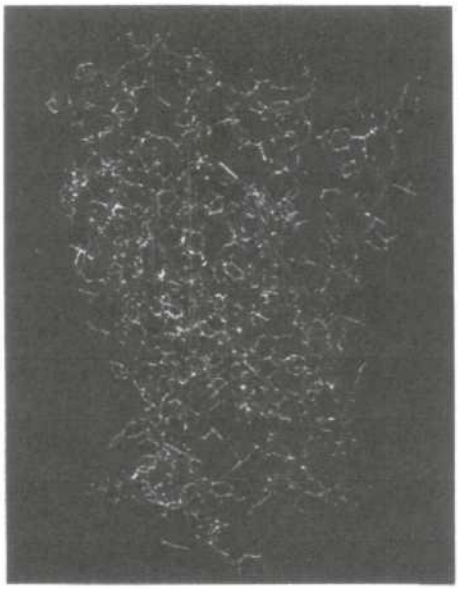
Bioinformatics methods: docking *in silico* on a molecular target of a cloned turkey beta-1-adrenoceptor (PDB ID 2vt4). Screening in silico consisted of four main stages: preparation of the ligand library, docking, evaluation of results and filtration procedures. The source of data on the structure of the receptor-antagonist complex - Protein Data Bank (PDB). The structure of the cloned mutant beta-1-adrenoceptor (ADRB1_MELGA) of turkey (*Meleagris gallopavo*) was selected as a model of the beta-1-adrenergic receptor. The amino acid sequence of the described ADRB1_MELGA receptor is 82% to the human beta-1 receptor and 67% to the human beta-2 receptor. The structure code of the ligand-receptor complex according to X-ray diffraction data is PDB code 2vt4, resolution 2,700 Å, R-factor = 0,215 R-free = 0,268 (1). Simulations were performed on chains A-D, amino acid sequence length 313 amino acids, molecular weight 148237.55 Da. The active center of the receptor is bounded by a cube with side 3.9 Å, the location of which is determined by the coordinates of the known cyanopindolol ligand in the selected crystal structure (X = 54.4123, Y = 33.595, Z = -11.6943) to construct mapenergetic potentials of atoms of 0.3 The ligand-binding pocket of the beta-1-adrenergic receptor contains 15 amino acid residues from 4 side chains of transmembrane alpha helices and 2 extracellular loops. These loops define the entrance to the ligand-binding pocket and are stabilized by two disulfide bonds and a sodium ion (2, 7, 10). The docking process used AutoDock Vina, integrated on the online mcule platform (http://doc.mcule.com/doku.php?id=dockingvina#docking_vina), which uses the Vina docking algorithm (9). For the docking procedure, the charges of receptor molecules and ligands were calculated by the Gasteiger-Marsili method (4) using the AutoDock Vina program. The search for the optimal geometry of the complexes was performed using a stationary active center and flexible ligands. The mobility of the latter was determined by the rotation around single bonds that are not part of the cycle. Next, we calculated the energy gain in the formation of the corresponding complex (EDoc, kcal/mol) - free binding energy to the corresponding site of the 2vt4 receptor at T = 298.15 K. For 3D visualization, we use the GLmol

browser based on WebGl (http://webglmol.osdn.jp/index-en.html). During the formation of the pose, the ligand was placed in the binding site, selecting its rotational and translational degrees of freedom. Subsequently, kinship is assessed on the basis of the formed posture. 4 attempts were made for each structure, and the most active position was considered to be the location of the ligand, which corresponds to the lowest energy (the largest gain in energy).

Results and discussion

The results of molecular docking of ademol and reference compounds to β1-adrenoceptors (PDB ID 2vt4,) are presented in tables 1-2 and in Fig. 2-5.

Figure 2. 3D-visualization of β1-adrenoceptor (PDB ID 2vt4).



Thus, the binding of ademol and its location in the hydrophobic pocket of the active site of the receptor is ensured by the formation of several key interactions with the docking energy EDoc = -7.5 kcal/mol (Fig. 2, 3). In particular, the most active position is the possibility of the formation of hydrogen bonds between the hydroxyl group of the propanamine fragment of ademol and the OH groups of threonine Trh80 and aspartic acid Asp83.

The tertiary amino group of the morpholine residue is able to form a hydrogen bond by interaction with the CONH2 group of asparagine. Hydrophobic interactions were recorded between the adamantane core of ademol and aliphatic residues of valine (Val243, Val64) and leucine Leu63.

Table 2. Molecular docking of ademol and reference compounds to β1-adrenoceptor (PDB ID 2vt4).

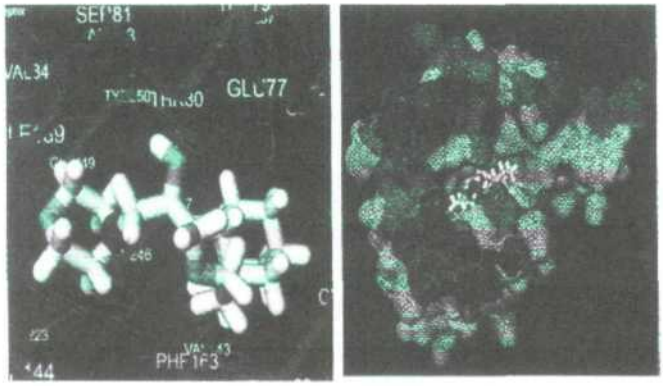
Compound	Docking indicators		Aminoacids of the active center of the beta-1 receptor, with which there is an interaction in position 1
	Position	Energy E_{Doc}, kcal/mol	
Ademol	1 2 3 4	-7.5 -7.4 -7.4 -7.4	Trh80, Asp83, Asn246, Phe163, Phe223, Trp247, Val243, Val64, Leu63
Propranolol	1 2 3 4	-7.9 -7.7 -7.6 -7.6	Trh80, Asp83, Asn246, Val84, Val87, Phe178, Phe224, Tyr250
Timolol	1 2 3 4	-7.0 -7.0 -6.9 -6.5	Trh80, Asp83, Tyr250, Asn246, Trp247, Val64, Val243.

Table 1. Comparative characteristics of descriptors and some properties of ademol molecule and β-blockers with phenoxypropanolamine structure.

Molecular descriptor or constant \ Molecule	Ademol (1)	Timolol (2)	Propranolol (3)	SR 59230A (4)
Log *K* (nM) relative to β-adrenergic receptors of the eye, *in vivo*	ND*	1.2	1.1	ND
Log $P_{o/w}$ (iLOGP)	1.73	2.77	3.23	3.83
Log $P_{o/w}$ (XLOGP3)	2.94	1.83	2.98	3.92
Log $P_{o/w}$ (WLOGP)	1.92	0.12	2.58	3.33
Log $P_{o/w}$ (MLOGP)	2.12	-0.36	2.35	3.08
Log $P_{o/w}$ (SILICOS-IT)	3.11	1.77	3.04	4.44
Consensus Log $P_{o/w}$** *in silico*	2.36	1.23	2.84	3.72
Log P, n-octanol/pH 7.4 buffer, *in vitro*	ND	1.91	3.21	ND
TPSA, Å²	41.93	107.98	41.49	41.49
Permeability through blood-brain barrier (method BOILED-Egg)**	+++	not	+++	+++
Bioavailability, *in silico*	+++	++	++++	+++
Bioavailability (%), *in vivo*	ND	60-90	≈100	ND
P- glycoprotein substrate *in silico*	not	yes	not	not
Solubility of the base in aqueous solutions Log *S* (SILICOS-IT), *in silico*	-2.94 soluble	-2.38 soluble	-3.51 moderately soluble	-6.64 poorly soluble
Molar mass, g/mol	323.47	316.42	259.34	325.44

Notes: *ND – *no data*; ** calculated *in silico on the SIB Swiss Institute of Bioinformatics platform (www.sib.swiss)*; *** *Calculated in silico by the method - A BOILED-Egg*

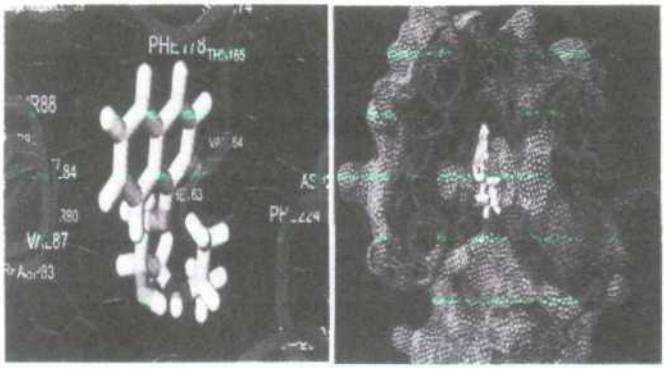
Figure 3. 3D-visualization of the binding of the ademol molecule with the docking index (EDoc = -7.5 kcal/mol) **with the active site of the β1-adrenoceptor** (PDB ID 2vt4) **by molecular docking.** Notes: A. Location of the ademol molecule relative to the amino acid residues of the active site receptor; B. Location of the ademol molecule in the active site of the receptor.



A. B.

Unlike ademol, propranolol contains an aromatic naphthalene nucleus, and therefore ϖ-ϖ interactions with phenylalanine residues (Phe178, Phe224) are additionally fixed for it, which obviously leads to increased binding to the receptor (EDoc = -7.9 kcal/mol).), although with very close values to ademol (Fig. 3, 4). The free OH group of propranolol, which is important for beta-blocking activity, also forms hydrogen bonds, like the ademol molecule, with the OH groups of the threonine residues Trh80, aspartic acid Asp83 in the active center of the receptor. The secondary amino group of the N-isopropylamine residue is able to form a hydrogen bond by interaction with the COH group of asparagine Asn246 and additionally with phenolic hydroxyl tyrosine (Tyr250). In addition, hydrophobic contacts of the propranolol molecule with valine residues are observed (Val84, Val87).

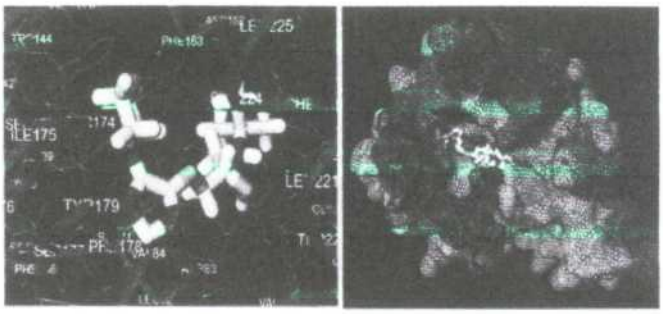
Figure 4. 3D-visualization of the binding of the propranolol molecule with the best docking index (EDoc = -7.9 kcal/mol) **to the active site of the β1-adrenoceptor** (PDB ID 2vt4) **by molecular docking.** Notes: A. Location of the propranolol molecule relative to the amino acid residues of the active receptor center; B. Location of the propranolol molecule at the active site of the receptor.



A. B.

According to the results of docking, Timolol had the lowest of the three drugs affinity for beta-1-adrenoceptor in terms of docking energy gain EDoc = -7.0 kcal / mol (Fig. 5). The free OH group of timolol in the most active position forms hydrogen bonds with the OH groups of threonine residues Trh80, aspartic acid Asp83 in the active center of the receptor and phenolic hydroxyl tyrosine (Tyr250). The secondary NH-amino group of the N-tert-butyl residue is able to form a hydrogen bond when interacting with the Ash246 Ash246 asparagine group and apparently creates some steric barriers due to bulk, which prevents the timolol molecule from entering the active receptor site more efficiently.

Figure 5. 3D-visualization of the binding of the timolol molecule at the best docking index (EDoc = -7.0 kcal/mol) **with the active site of the β1-adrenoceptor** (PDB ID 2vt4) **by molecular docking.** Notes: A. Location of the timolol molecule relative to amino acid residues active receptor center; B. Location of the timolol molecule at the active site of the receptor.



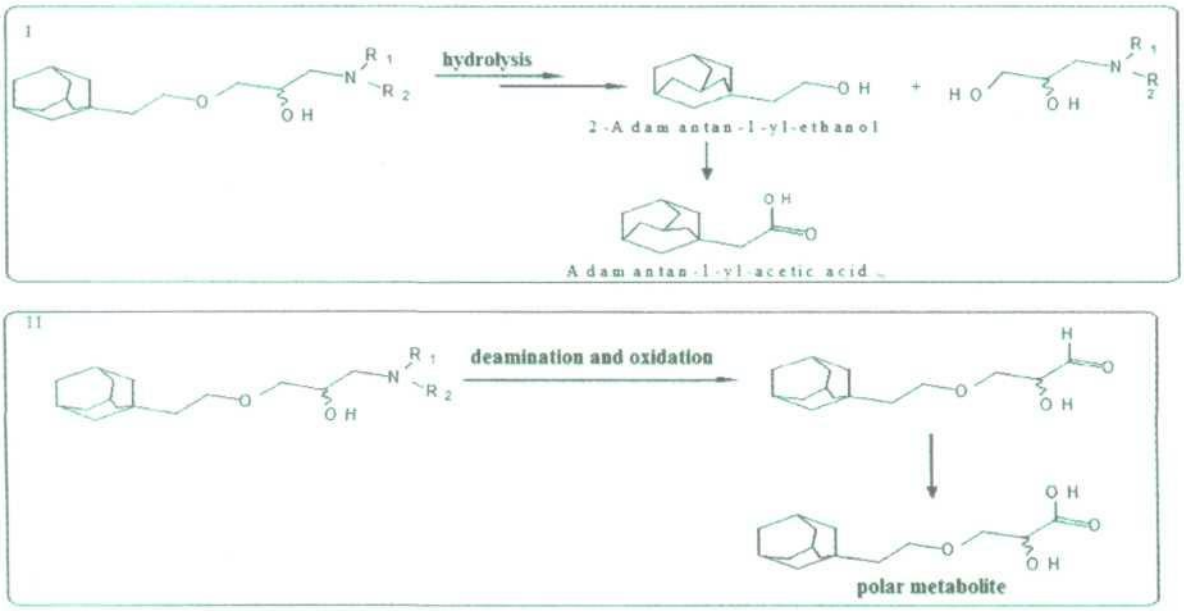
A. B.

Analyzing the peculiarities of the chemical structure of ademol, it should be noted that in the alkoxy group it contains 1-adamantylethyl substituent, and as an amine fragment - morpholine. In fig. 6 shows the probable pathways of ademol metabolism on the principle from less polar to more polar soluble metabolites. Although a simple ester bond is quite strong to metabolism, path (I) seems, in our opinion, more likely. Ademol metabolism can also occur due to the side chain.

Using the method of molecular docking (Tables 1, 2, Fig. 2-6), the affinity of atenolol for the beta-1-adrenergic receptor was evaluated in comparison with propranolol and timolol. The results indicate the ability of ademol to bind to the selected target, not inferior to timolol and close to propranolol.

Analyzing the data in table 1 and Fig. 1 and 5 it is possible to identify and predict probable ways of chemical modification of the ademol molecule and what functional groups are necessary for introduction into this structure to enhance affinity for various NMDA receptor sites with the subsequent purpose of synthesis of a more effective neuroprotector. Comparing the structure of ademol and amantadine (1-adamantylamine), it should be noted that both drugs are weak antagonists of glutamate NMDA receptors and inhibit

Figure 6. Probable pathways of ademol metabolism.



the generation of impulses in the motor neurons of the CNS (6). For them, it can be assumed that the introduction of NH2-amino group will enhance the described type of pharmacological and receptor activity. In support of the thesis regarding the presence of a free amino group directly associated with the adamantane framework indicate data on the drug from the group of amino-adamantanes - midanthan, which has even greater than Mg2 + ions tropism to the receptor channel, and blocking processes associated with the entry of calcium ions into the channel. It can also be recommended to introduce groups that will hypothetically chelate Mg2 + ions and prevent them from entering the NMDA receptor channel.

Conclusions

1. By the method of molecular docking in silico on the cloned β1-adrenoceptor Meleagris gallopavo, the ability of ademol to block β-adrenoceptors was established. Depending on the cerebrospinal fluid activity and affinity for β1-adrenoceptors (Edoc, kkal / mol) ademol occupies an intermediate position between propranolol and timolol.
2. The metabolism of ademol can occur both through the side chain with the formation of its metabolite adamantyl-1-yl-acetic acid and 2-adamantanthyl--1-ethanol excreted in the urine, and due to the binding of ademol to plasma albumin, it provides an alternative pathway of metabolism that occurs slowly in the liver due to the presence of an inert adamantane radical.
3. The introduction of NH2-amino group, glycine or glutamic acid residues into the framework of the ademol molecule, will increase its affinity, which will enhance the neuroprotective activity through a more complete and physiological blockade of NMDA receptors. Ademol is a promising neuroprotective agent that can be used in brain injury, which is associated with increased intracranial pressure (TBD).*

*Conflicts of Interest. The authors declare that there is no conflict of interest.

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