AN UNCONVENTIONAL «DOCKING-ASSISTED» APPROACH TO CLARIFYING THE MECHANISM OF ACTION OF PROMISING ANTIDEPRESSANT ATRISTAMINE

Illia Podolskyi, associate professor, National University of Pharmacy, Kharkiv, Ukraine, illya.podolsky@nuph.edu.ua

Marharyta Suleiman, associate professor, National University of Pharmacy, Kharkiv, Ukraine, suleiman.nfau@outlook.com

Lina Perekhoda, professor, National University of Pharmacy, Kharkiv, Ukraine, linaperekhoda@ukr.net

Introduction

The new promising substance – 2-methyl-3-(phenylaminomethyl)-1H-quinolin-4-one (laboratory name «Atristamine») is now extensively studied in the National University of Pharmacy (Kharkiv, Ukraine) (fig. 1).

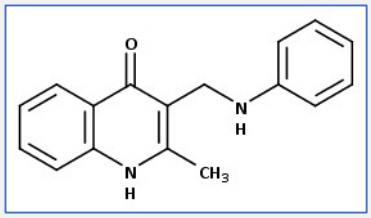

Figure 1. The structural formula of 2-methyl-3-(phenylaminomethyl)-1H-quinolin-4-one (Atristamine).

Atristamine exhibits excellent antidepressant properties on the experimental models of depression [1, 2]. It also shows the anti-amnesic, learning enhancing, cerebroprotective, antihypoxic, actopropective and analgesic activities [3-6].

Ascertainment and studying the mechanisms of the pharmacological action of promising drug candidates is one of the most important stages in the study of new molecules.

Previously, using ELISA method it has been found that a reliable decrease in the concentration of serotonin (–16,8%, $p<0,05$) was consistent with the increased levels of dopamine (+22,0%) and epinephrine (+13,0%) in the brain of mice after administration of Atristamine in the dose of 100 mg/kg [7]. Thus, pharmacological effects of Atristamine were associated with these changes. Different effects of Atristamine on the neurotransmitter systems of the brain have been already discussed in the previous studies [8]. For example, it has been shown that Atristamine in the dose of 100 mg/kg has slight influence on the GABA-ergic system (on the models of thiosemicarbazide-induced seizures and thiopental-induced narcosis in mice), glycine-ergic structures (on the model of strychnine-induced seizures in mice) and mildly modulates the purinergic system (decreases the anxiogenic effect of caffeine in the open field test in mice) [8]. Furthermore, interrelations between effects of Atristamine and serotonin, dopamine and norepinephrine neurotransmitter systems *in vivo* using the corresponding pharmacological analyzers have been studied [9]. It has been concluded, that the antidepressant action of Atristamine may be explained predominantly by its complicated influence on the serotonin, dopamine and norepinephrine neurotransmitter systems.

At some point, it became clear that the pharmacological effects of Atristamine are largely intertwined, complementing each other. Thus, it was decided that *in silico* methods can be useful in order to generalize results and clarify the question about mechanisms of action of Atristamine.

Materials and methods

The data of pharmacological experiments served as the basis for the selection of suitable targets for docking studies. The active centers of the following macromolecules from Protein Data Bank were selected as biological targets for docking: serotonin transporter (SERT, PDB ID: 5I6X), dopamine transporter (DAT, PDB ID: 4M48), leucine transporter (LeuTAA, PDB ID: 2A65), dopamine D_2 receptor (D2R, PDB ID: 6CM4), serotonin 5-HT_{1B} receptor (5-HT1BR, PDB ID: 5V54), serotonin 5-HT_{2A} receptor (5-HT2AR, PDB ID: 6A94), serotonin 5-HT_{2B} receptor (5-HT2BR, PDB ID: 6DRX), serotonin 5-HT_{2C} receptor (5-HT2CR, PDB ID: 6BQH). For each target, molecules of appropriate inhibitors (for transporters), agonists and antagonists (for receptors) were additionally docked. Considering the possible tautomerism of the Atristamine molecule (oxo- and hydroxy- forms), docking studies were performed for both tautomers.

For receptor-oriented flexible docking, the Autodock 4.2 software package was used. Ligands were prepared using the MGL Tools 1.5.6 program. The Ligand optimization was performed using the Avogadro program. To perform calculations in the Autodock 4.2 program the output formats of the receptor and ligand data were converted to a special PDBQT format. The receptor maps were made in MGL Tools and AutoGrid programs. Water molecules, ions, and the ligand were removed from the PDB file. Indicators of the efficiency of binding of ligands to targets are scoring function (Affinity DG) for the best conformational positions, the values of the free binding energy and inhibition constants (EDoc kcal/mol and Ki uM (micromolar)). The character and number of bonds formed between the ligand and the active site of the macromolecule were analyzed visually using 2D-diagrams and 3D-models constructed. The visual analysis was performed using the Discovery Studio Visualizer program.

Result and discussion

Receptor-oriented flexible docking has been used to identify probable targets by which Atristamine may realize its pharmacological activity. It should be noted that the method was used unconventionally. Typically, a wide range of compounds are tested relative to a single target and the best binding parameters are selected. In our case, Atristamine was studied, having experimentally confirmed data on pharmacodynamic effects, in order to identify probable targets that can provide the corresponding mechanisms.

The docking results (tab. 1) show that Atristamine has the best interaction with the serotonin transporter (SERT, PDB ID: 5I6X): in oxo form it has the lowest binding energy, which exceeds that of citalopram and paroxetine.

Table 1. The most promising docking results for Atristamine (LEUTAA and SERT).

Compound	Free binding energy, kcal/mol	Inhibition constant (Ki), uM	Scoring function (Affinity DG)	The character of hydrogen bond, lenght, Å
LEUTAA (2A65)				
Atristamine (oxo)	-5,69	67,41	-8,0	ASP404 (4,76); ARG30 (4,32)
Atristamine (hydroxy)	-5,01	213,83	-8,2	ASP404 (4,53 – OH); ASP404 (4,70 – NH); ARG30 (4,55)
Atomoxetine	-4,97	226,67	-7,4	–
SERT (5I6X)				
Atristamine (oxo)	-8,14	1,07	-9,1	THR497 (2,50); PHE335 (2,23)
Atristamine (hydroxy)	-6,88	9,05	-9,1	TYR95 (2,32)
Citalopram	-6,63	13,83	-9,5	THR497 (4,60)
Paroxetine	-6,83	9,93	-10,6	ASP98 (4,49)

Furthermore, visual analysis of the target ligand complex showed the formation of two hydrogen bonds between the phenylalanine residue (335) and the NH group of the quinolone ring (interatomic distance – 2.23 Å) and the threonine fragment (497) and the keto group of the heterocycle (interatomic distance – 2.50 Å) (fig. 2). It is the possibility of forming hydrogen bonds that testifies to the energy efficiency and stability of the formed complex.

It is shown that the complex is additionally stabilized by three π-π-interactions (fig. 2): phenylalanine (335) simultaneously binds to both the phenyl substituent in the phenylaminomethyl moiety and the heterocyclic quinolone ring, and the phenylalanine residue (341) interacts with the aromatic moiety. Valine (501) forms a π-alkyl bond with the aromatic ring of the heterocycle.

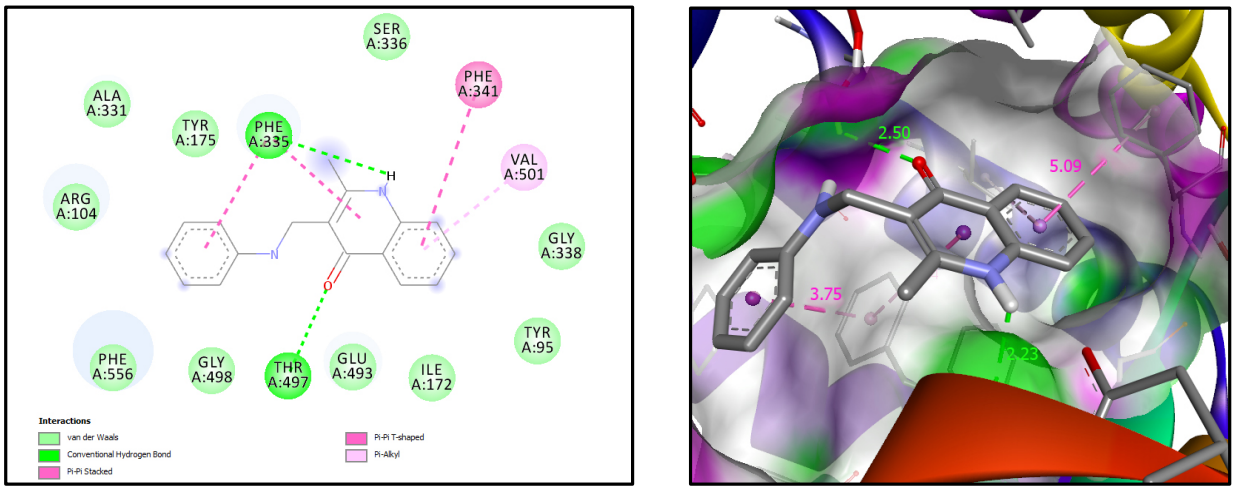

Figure 2. 2D-diagram and 3D-model of complex «Atristamine in oxo form – active site of serotonin transporter (SERT)».

The predominant effect of Atristamine on neurotransmitter transporters is supported by the results of docking to the leucine transporter (LEUTAA, PDB ID: 2A65), which until recently was used as the only model for the study of norepinephrine, serotonin and dopamine reuptake inhibitors. The calculated binding energy and inhibition constant (tab. 1) are better compared to the selective inhibitor atomoxetine.

Conclusion

The obtained results show that Atristamine can realize both the main antidepressant effect and additional types of biological action *via* binding to neurotransmitter transporters (serotonin, norepinephrine and dopamine), which leads to inhibition of their reuptake. At the same time, direct interaction of Atristamine with receptors cannot be ruled out. This is supported by a comparison of the binding energies of the test molecule and known ligands with the corresponding targets.

Furthermore, the above study is a good example of an unconventional approach to clarifying the mechanisms of the pharmacological action of a new substance using *in silico* methods.

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