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ANOTHER LOOK AT THE BIOLOGICAL ROLES OF A PLANT ALKALOID-BERBERINE

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Abstract: For millennia, berberine extracts or berberine itself has been the effective traditional drug with wide application due to its broad spectrum of antibiotic activity. A significant aspect of the berberine's physiological activity that is often overlooked is the ability to go through the blood-brain barrier and has an impact on different processes and irregularities in the brain such as dementia and Alzheimer's disease. Potential inhibitory activity towards enzymes for which is believed to be involved in these diseases, in this paper is confirmed by molecular docking simulations. Binding energies suggest that berberine exhibits good potential inhibitory activity and confirms that one of the aspects of suppression of Alzheimer's disease and dementia is the inhibition of cholinesterase enzymes.

Keywords: Berberine, Molecular docking, Alzheimer's disease, Plant-based drugs, Traditional medicine

Introduction

Berberine, is an isoquinoline quaternary alkaloid extracted from the Chinese herbs such as *Hydrastis canadensis, Berberis aristata, Coptis chinensis, Coptis rhizome, Coptis japonica, Phellondendron amurense, Phellondendron chinense schneid* and a variety of other plants in the *Berberidaceae* and *Ranunculaceae* families. It has a long history of usage in traditional Chinese medicine. Berberine can be found in the different parts of plant organisms and is a product of various metabolic processes with a wide range of biological and physiological properties.

Berberine's various pharmacological properties suggest that this alkaloid has considerable potential as a medication in a wide range of clinical applications. In this regard, berberine's structure (Figure 1) represents a biologically significant skeleton as well as an appealing natural lead molecule for the insertion of numerous chemical alterations in appropriate spots in the hunt for more selective and more specific medical applications.

For millennia, berberine extracts or berberine itself has been the most effective traditional cure in China for treating dysentery and infectious diarrhea due to its broad spectrum of antibiotic activity. Berberine has antibacterial properties because it can accumulate in bacterial cells and bind to single-stranded and double-stranded DNA, causing bacterial death by DNA damage (Boberek et al.,

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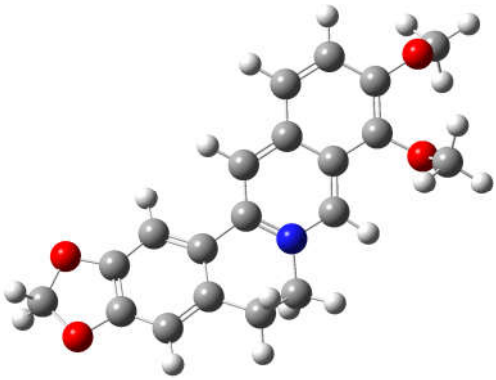
2010). It has poor efficacy against Gram-negative bacteria (Boberek et al., 2010), but the MDR pump NorA inhibition makes it more effective against Gram-positive bacteria, such as Mycobacterium TB and MRSA (Methicillin-Resistant Staphylococcus aureus) (Boberek et al., 2010; Samosorn et al., 2009). It also expressed antifungal properties against *Aspergillus niger, Penicillium niger, Candida albicans,* and *Cryptococcus* (Imanshahidi and Hosseinzadeh, 2008; Vuddanda et al., 2010) Berberine inhibits the synthesis of tumour necrosis factor-a (TNF-a), interleukin-6 (IL-6) and monocyte chemoattractant protein 1 (MCP-1) and so has anti-inflammatory properties (MCP-1). Through the mitogen-activated protein kinase (MAPK) and NF-kB signalling pathways, it also impacts prostaglandin E2 (PGE2) synthesis and exudate formation, as well as down-regulating the expression of COX-2, matrix metalloproteinase (MMP)-2 and (MMP-9) (Remppis et al., 2010).



Slika 1. Optimizovana struktura berberina
Figure 1. Optimized structure of berberine

Beside antimicrobial and antiviral effects, berberine has been found to be effective in the treatment of type 2 diabetes (Yin et al., 2008; Hui et al., 2009). This benefit was shown to be mediated by improved glucose homeostasis, higher insulin expression, pancreas beta cell regeneration, and lower lipid peroxidation experimentally induced in diabetic rats (Chen et al., 2011; Hui et al., 2009). After administering berberine to individuals with metabolic syndrome, promising outcomes were achieved, with insulin sensitivity improved and therefore favourable effects on the disease.

It is well known that the majority of drugs with anti-tumour properties derives from natural compounds (Tan et al., 2011). In this context, it is important to emphasize that recent studies have shown that berberine exerts *in vitro* anti-proliferative effects on different cancer cell lines (Sun et al., 2009; Tan et al., 2011).

One aspect of the berberine's physiological activity that is often overlooked is the ability to go through the blood-brain barrier and has an impact on different processes and irregularities in the brain such as dementia and Alzheimer's disease.

Berberine expresses these properties in four different aspects:

1. Antioxidative activity of berberine
2. Anti-inflammatory properties of berberine
3. Anti-cholinesterase activity of berberine
4. Anti-amyloid activity of berberine

In this paper, anti-cholinesterase activity of berberine was investigated by molecular docking simulation methods.

Material and methods

The geometries of the tested compound were optimized using the Density Functional Method (DFT) method M06-2X with basis set 6-311G (d, p). (Zhao and Trulhar, 2008; Check et al., 2001) Optimization of ligand structure was performed using the Gaussian 09 software package (Frisch et al., 2010).

The SwissTargetPrediction server was utilized to predict relevant protein targets with which investigated compound might interact. (Gfeller et al., 2014)

AutoDock 4.0 software package with implemented AMBER force field was used to predict the location and energy of noncovalent interactions of ligands with proteins. The monomeric crystal structures of the proteins in PDB format (PDB: 6EMI, 5HF6) were taken from the RCSB Protein Data Bank website. A program called Discovery Studio 4.0 was used to prepare the structures for docking. This program was also used to visualize the obtained results, as well as to analyze the interactions between proteins and ligands. Using programs and modules implemented in AutoDockTools (ADT), polar hydrogen was added to the protein. In addition, partial atomic charges were attributed to atoms in the protein using the Kollman model, which is based on quantum mechanical calculations of the charges of atoms within amino acids. The active binding sites of the test compounds were determined using AGFR. As maps for each ligand atom are necessary for efficient docking with AutoDock, AutoGrid was used for the purpose of their calculations. Using Auto Grid, the coordinates of the active site were determined, which will occupy a ligand during docking. The Lamarckian Genetic Algorithm (LGA) was used to generate ligand orientation within the active site. (Morris et al., 1998)

To predict the binding affinity of a ligand for a protein, AutoDock uses empirical functions based on free binding energies (ΔG_{bind}). (Huey et al., 2007) Free binding energies include several different components with different effects. These components represent different influences that interact between proteins and ligands, as shown in the following equation:

$$\Delta \mathbf{G}_{\text{bind}} = \Delta \mathbf{G}_{\text{vdw+hbond+desolv}} + \Delta \mathbf{G}_{\text{elec}} + \Delta \mathbf{G}_{\text{total}} + \Delta \mathbf{G}_{\text{tor}} - \Delta \mathbf{G}_{\text{unb}} \quad (1)$$

where $\mathbf{\Delta G_{total}}$ represents the energy of the whole system, $\mathbf{\Delta G_{tor}}$ represents the torsion energy, $\mathbf{\Delta G_{unb}}$ represents the energy of non-covalent interactions inside the system, $\mathbf{\Delta G_{elec}}$ represents the electrostatic energy and $\mathbf{\Delta G_{vdw+hbond+desolv}}$ represents the sum of Van der Waals, hydrogen bond and desolvation energies (Morris et al., 1998)

Results and discussion

SwissTargetPrediction server estimated that berberine will exhibit good inhibitory potency towards a dozen of different proteins, but the highest score was obtained for Acetylcholinesterase (AChE), and its analogue Butyrylcholinesterase (BChE). These proteins are found to be one of the main targets when it comes to designing a drug against Alzheimer's disease and dementia. Through the years, a large number of different inhibitors of these enzymes was discovered, even though high inhibitory activity against these enzymes maybe helps with lowering symptoms of some diseases, it causes a large number of unwanted side effects. For this reason, the discovery of naturally occurring inhibitors, especially compounds with proven biological potency towards these types of diseases is the aim of research in this field for the last couple of decades. As previously mentioned, there are different aspects of the action of berberine when it comes to Alzheimer disease, and anti-cholinesterase activity is in correlation with targets predicted by SwissTargetPrediction server.

Molecular docking simulations performed in this paper suggest that berberine exhibits good potential inhibitory activity towards both enzymes, with medium to high binding potential. Parameters obtained by molecular docking simulations are presented in Table 1.

Tabela 1. Parametri koji opisuju interakcije berberina i AChE i BChE
Table 1. Parameters which describe the interactions of berberine and AChE and BChE

Ligand-Enzyme Complex	ΔG_{bind} (kcal mol^{-1})	K_i (μM)	ΔG_{inter} (kcal mol^{-1})	ΔG_{elec} (kcal mol^{-1})	ΔG_{total} (kcal mol^{-1})	ΔG_{tor} (kcal mol^{-1})
Berberine-AChE	-8.20	0.97	-8.80	+0.01	-0.31	0.60
Berberine-BChE	-8.00	1.37	-8.59	-0.10	-0.29	0.60
Donepezil-AChE	-9.47	0.11	-11.26	-0.44	-0.86	1.79
Donepezil-BChE	-8.77	0.37	-9.55	-1.01	-0.75	1.79

As can be seen from Table 1, a low inhibitory constant indicates that relatively low concentrations of berberine are necessary for enzyme inhibition. Donepezil (2-((1-Benzylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one) was used as standard for AChE and BChE inhibition. However, even though donepezil shows slightly better inhibitory activity towards AChE and BChE, it has a list of negative side effects and is not suitable for preventive use, while berberine is a natural compound, and has a lot less side effects. It should be emphasized that the anti-cholinesterase activity of berberine is only one aspect of berberine's activity towards Alzheimer's disease. Bearing in mind that all aspects are synergetic and that berberine has a wide range of beneficial physiological effects, the usage of berberine or its derivatives as a drug against Alzheimer's disease should be further investigated in future.

Conclusion

Berberine is a naturally occurring compound found in different plant species with a wide range of biological and physiological roles. It was used in traditional medicine for centuries and it was found to be very successful in the treatment of different diseases. One overlooked role of berberine is its potential in the treatment of Alzheimer's disease and dementia. In this paper, one of the four aspects of berberine's action towards the treatment of these two diseases was investigated. It was found that berberine exhibits good inhibitory activity towards AChE and BChE, enzymes for which is believed to participate in the development of Alzheimer's disease and dementia. Berberine and its derivatives should be studied further as a potential treatment for dementia and Alzheimer's disease.

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JOŠ JEDAN POGLED NA BIOLOŠKU ULOGU BILJNOG ALKALOIDA BERBERINA

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Apstrakt: Već milenijumima, ekstrakti berberina ili sam berberin je bio efikasan tradicionalni lek sa raznovrsnom primenom usled širokog spektra delovanja antibiotika. Značajan aspekt fiziološke aktivnosti berberina koji se često zanemaruje je sposobnost prolaska kroz moždanu barijeru i uticaj na različite procese i nepravilnosti u mozgu kao što su demencija i Alchajmerova bolest. Potencijalna inhibitorna aktivnost prema enzimima za koje se veruje da su uključeni u ove bolesti, u ovom radu je potvrđena molekulskim dokingom. Energije vezivanja pokazuju da berberin ispoljava dobru inhibitornu aktivnost i potvrđuje da je jedan od aspekata supresije Alchajmerove bolesti i demencije inhibicija enzima iz grupe holinesteraza.

Ključne reči: Berberin, Molekulski doking, Alchajmerova bolest, Lekovi na bazi biljaka, Tradicionalna medicina