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Evaluation of berberine inhibitory effects on influenza neuraminidase enzyme: A molecular dynamics study

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ABSTRACT

Introduction: Due to the high prevalence and drug resistance reported for the influenza virus in recent years, much research is being conducted on the discovery and introduction of more effective drugs against the virus. In this regard, the present bioinformatics study examined the inhibitory effects of berberine, a plant-based alkaloid, on influenza virus neuraminidase using docking and molecular dynamics studies.

Methods: To conduct this study, the three-dimensional structure and PDB file of influenza virus neuraminidase were prepared from the protein and molecular information database, and the structure file of the berberine and oseltamivir (as positive control) molecules were prepared from the PubChem database. Using GROMACS software, simulation and molecular dynamics calculations were performed in the absence of an inhibitor. Molecular docking studies were performed using AutoDock software, and re-simulation of the protein-ligand complex was performed using GROMACS software.

Results: Berberine was bound to the neuraminidase molecule with three hydrogen bonds and eleven hydrophobic bonds at the binding site. The amount of binding energy (BE) of berberine and oseltamivir was equal to -7.93 and -6.27 kcal/mol with the estimated inhibition constant (EIC) of 1.5 and 25.2 µM, respectively. Over simulation time, the radius of gyration (Rg) of the enzyme at berberine binding increased, but there was no significant difference in system energy changes (TE).

Conclusion: Due to berberine binding, structural changes occur in the secondary and tertiary structures of influenza virus neuraminidase. The large number of created bonds, the low level of binding energy, and the low concentration of the EIC indicate the high tendency of berberine to bind to the binding site of neuraminidase.

Implication for health policy/practice/research/medical education:
The results of this study can be used for the appropriate understanding of the drug's effect mechanism on the virus neuraminidase from the perspective of molecular dynamics and assist researchers in the design and production of anti-influenza drugs.

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Introduction

Influenza is an acute respiratory disease caused by infection with the influenza virus. Influenza virus belongs to RNA viruses and is a member of the Orthomyxovirus family. Based on the core protein of the virus, it is divided into four types, A, B, C, and D, which are different from each other in terms of genome structure and pathogenicity. Influenza A virus causes pneumonia and severe complications such as acute respiratory pain syndrome, due to which 250 000 to 500 000 people die per year worldwide (1). Influenza virus has eight genome fragments of single-stranded RNA with negative polarity,

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which encode at least 11 proteins (2,3). The surface proteins of the influenza virus are hemagglutinin and neuraminidase, which play a role in the entry, spread, and pathogenesis of the virus and are the main targets of the immune system. In this regard, many antiviral drugs that can target and inhibit these enzymes have been produced. Among the neuraminidase inhibitors, we can mention drugs such as zanamivir, oseltamivir, laninamivir, and peramivir, which, while being effective, may cause certain side effects and resistance in strains. Neuraminidase is a complex multifunctional protein of the virus, whose main function is in the final stage of infection where it removes sialic acids from cell receptors and causes the release of produced viruses; therefore, it is the main target of current antiviral treatments (4).

Because available chemical drugs cause side effects, and resistance to a large number of them has been reported, their use is limited. Therefore, efforts are directed toward the production of more antiviral drugs, especially drugs of herbal origin (5).

In this regard, the anti-influenza effects of various compounds such as polyphenols, flavonoids, saponins, glycosides, and alkaloids, isolated from a number of medicinal plants, have been widely studied, and it has been shown that these compounds produce inhibitory effects on binding, absorption, proliferation or maturation of this type of virus (6,7).

Berberine is known as the most important alkaloid in the root and bark of barberry (*Berberis vulgaris*). The effects reported for barberry are mostly related to this alkaloid (8). Previous studies have shown that compounds of natural origin such as berberine can inhibit the influenza virus (9). Alkaloids produce anti-influenza effects by inhibiting neuraminidase and preventing the integration of the viral envelope and cell membrane (10). Among the compounds identified in that study, berberine exhibited strong inhibitory effects against viral neuraminidase, in addition to its anti-influenza effects (11). In other studies, the antiviral effects of berberine on other human pathogenic viruses such as herpes simplex virus, viruses involving the respiratory system, and cytomegaloviruses have been noticed (12,13).

Nowadays, the use of molecular dynamics simulations and in situ studies can provide useful information regarding the prediction of the functioning of enzymes and proteins in exposure to therapeutic drugs. In addition to showing the degree of binding of the drug to the binding site, these studies can identify the amino acids at the binding site of the enzyme and specifically determine the factors related to the molecular dynamics of the enzyme before and after the binding of the drug to the binding site and the differences that are created. Given the importance of neuraminidase for inhibiting the activity of the influenza virus, this study investigated the inhibitory effects of berberine on the neuraminidase of the influenza virus using docking and molecular dynamics studies.

Materials and Methods

Preparation of molecular structures

The PDB file of the viral neuraminidase protein was prepared from the protein database (PDB) at https://www.rcsb.org (PDB code 7NN9). Ligands and other water molecules were removed from the protein structure and the energy level of the protein structure was stabilized using ArgusLab software. Also, molecular information and structural file of berberine (PubChem CID: 2353) and oseltamivir (PubChem CID: 65028) molecules were obtained from the PubChem database and converted to PDB file using Avogadro software, and their energy was optimized.

Protein molecular dynamics simulation

The studies of the molecular dynamics' simulation of the viral neuraminidase protein were first performed in water and salt until the relevant structures were subjected to changes in temperature, pressure, and concentration of 140 mM and reached the equilibrium state.

Viral neuraminidase protein was simulated using GROMACS 4.6.1 software and G43A1 force field in an aqueous solvent at a time interval of 10 nanoseconds. In this study, the SPC216 model was used (14). In order for the system to reach a concentration of 140 mM, the required number of Na and Cl ions was added instead of the solvent. To perform the simulation, the steepest descent algorithm was used. Then the balancing steps were done by means of NVT and NPT ensemble by using the LINCS algorithm to integrate 50 000 steps. The paths saved in the simulation were used as controls to analyze the structural parameters of the viral neuraminidase protein in the absence of the ligand (15).

Molecular docking

Molecular docking studies were performed using AutoDock version 4.2 software in the Linux operating system. After generating PDBQ and PDBQT files for berberine herbal alkaloid and oseltamivir as ligands, as well as PDBQ and PDBQT files for viral neuraminidase protein, molecular docking for ligands was performed 200 times separately. The obtained information was analyzed in the n.dlg text file. Discover Studio software was used to determine the number of hydrogen and hydrophobic bonds between the viral neuraminidase protein and ligands, as well as to determine the type and number of amino acids involved in the binding site. After obtaining the docking results, molecular dynamics steps of viral neuraminidase protein were performed in the presence of ligands.

Molecular dynamics simulation of protein-ligand complex

In this step, molecular dynamics simulation of the

viral neuraminidase protein complex to berberine and oseltamivir was performed in an aqueous environment as per the above method. The results of the simulation of viral neuraminidase protein were compared to the results of the simulation of the protein-ligand complex using Ghrapher 10 software (14).

Results

According to the molecular docking results, there were 17 bonds between the enzyme and berberine at the binding site. Berberine formed hydrogen bonds with three amino acids Asn417, Glu385, Asn386 and 14 non-hydrogen bonds with amino acids Tyr370, Glu369, Val383, Phe362, Leu371, His346, Ala402, Gly404, Ile416, Thr400, Asn401, Arg384, Tyr320, and Asn448. It should be noted that all these amino acids are located in the catalytic regions of neuraminidase. The BE (estimated free energy of binding) of neuraminidase and berberine was equal to -7.93 kcal/mol. In addition, the estimated inhibition constant (EIC) was calculated at 1.5 µM (Table 1 and Figure 1). Besides this, the BE of neuraminidase and oseltamivir (as positive control) was equal to -6.27 kcal/mol (Table 1 and Figure 1).

The results of molecular dynamics studies and the amount of RMSD changes showed that the neuraminidase molecule reached stability in both without binding to berberine and binding to berberine after 5 ns, and from 5 ns to 10 ns, the amounts of molecules' fluctuations minimized and the simulation system remained stable (Figure 2A).

The results of radius of gyration (Rg) calculations showed that the neuraminidase molecule reached stability in both without binding to berberine and binding to berberine over the simulation time. Over this time, the Rg of the enzyme in the state of berberine binding increased (2.13 ± 0.01 and 2.19 ± 0.01 nm before and after docking, respectively). This increase in the Rg indicates the structural changes caused by the binding of berberine to neuraminidase, which can affect the function of the enzyme (Figure 2B).

System total energy (TE) changes for the neuraminidase molecule in both without binding to berberine and binding to berberine during simulation are shown in Figure 2C. It seems that the binding of berberine to neuraminidase does not have much effect on the total energy changes of the system.

Regarding fluctuations in each amino acid (root-mean-square fluctuation, RMSF) related to the binding of berberine to the viral neuraminidase enzyme, a sharp decrease in the fluctuations in amino acids 374-379 (Tyr374, Asp375, Ser376, Ser377, Gly378, Lys379) and a sharp increase in the fluctuations in amino acids 352-358 (Gly352, Phe353, Trp354, Asn355, Ala356, Gly357, Leu358) were observed (Figure 2D).

Discussion

Numerous plant-derived products, including polyphenols, flavonoids, alkaloids, and lignans, have shown promising results as adjunctive or alternative medicines to available influenza treatments (16-18). In this regard, the anti-influenza effects of various compounds such as polyphenols, flavonoids, saponins, glycosides, and alkaloids isolated from a number of medicinal plants have shown that these compounds produce inhibitory effects on binding, absorption, proliferation, or maturation of this virus (6).

The results of this study showed that berberine could inhibit neuraminidase protein by binding to its active site. Berberine with a binding energy of -7.93 kcal/mol showed more activity against neuraminidase than the reference drug oseltamivir. Based on the same method, the binding energy of oseltamivir was calculated as -27.6 kcal/mol. According to the molecular docking results of berberine with neuraminidase protein, it was determined that its non-polar parts (aromatic rings) mainly show hydrophobic interactions with the nonpolar parts of the protein, including amino acids Tyr370, Glu369, Val383, Phe362, Leu371, His346, Ala402, Gly404, Ile416, Thr400, Asn401 and hydrogen interactions with amino acids Asn448, Glu385, and Asn386.

The results of the present study also showed that berberine could directly bind to the active site of the neuraminidase enzyme, which can disrupt the enzyme's function. The EIC represents the minimum concentration of berberine that is necessary for binding to neuraminidase, which was about 1.5 µM in our study. This low concentration of the drug along with the low level of binding energy indicates the high tendency of berberine to bind to the binding site of neuraminidase.

Table 1. The results of molecular docking and the amount of binding energy in the binding sites of berberine and the ligands

Ligand	PubChem CID	BE kcal/mol	FIE kcal/mol	EIC µM	Bonds	
					Hydrogen bonds	Non-hydrophobic bonds
Berberine	2353	-7.93	-8.53	1.53	Asn417, Glu385, Asn386	Tyr370, Glu369, Val383, Phe362, Leu371, His346, Ala402, Gly404, Ile416, Thr400, Asn401, Arg384, Tyr320, Asn448
Oseltamivir	65028	-6.27	-8.96	25.2	Thr288, Glu283, Gly357	Glu286, Asp356, Val358, Leu383, Thr384, Thr360, Arg304

BE: Estimated free energy of binding (kcal/mol); FIE: final intermolecular energy (kcal/mol); EIC: estimated inhibition constant (µM).

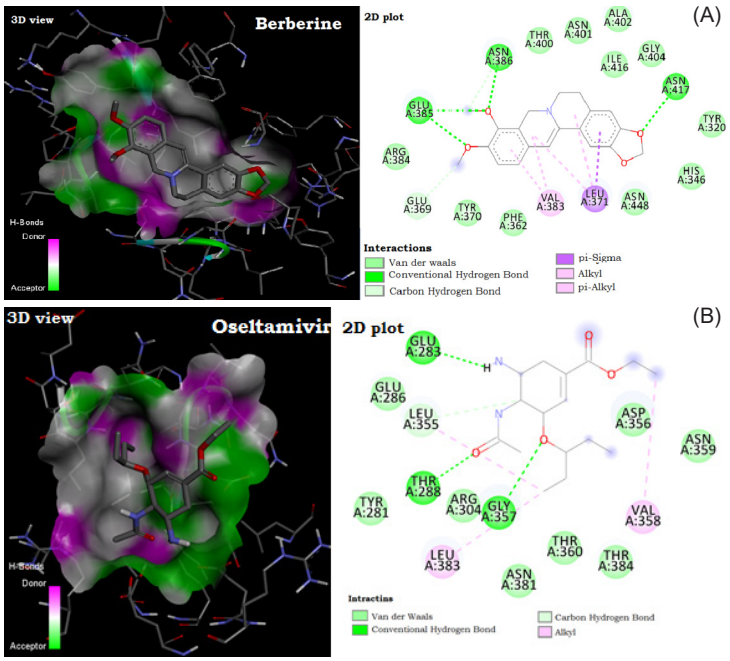

Figure 1. Stimulation figures of viral neuraminidase amino acids involved in interactions with berberine and oseltamivir. A: Interaction with berberine; B: Interaction with oseltamivir.

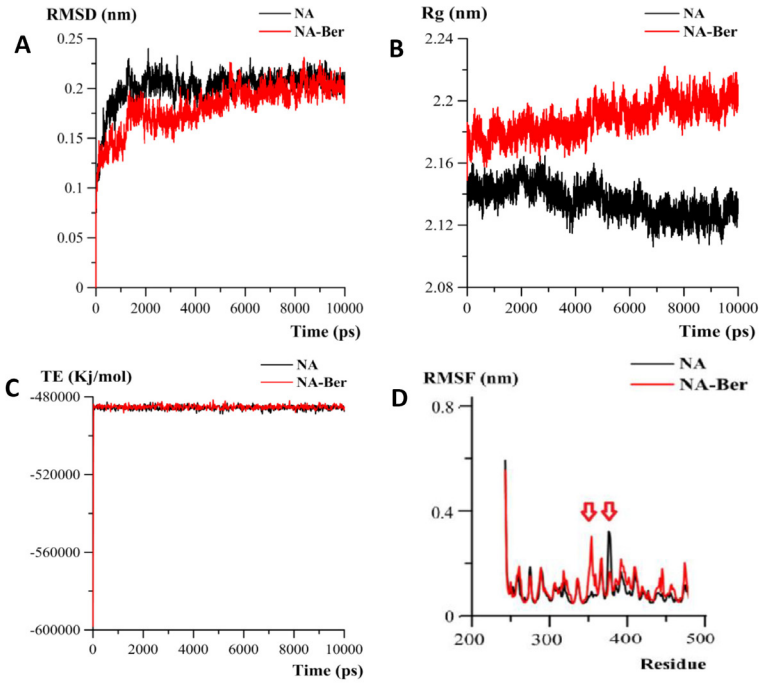

Figure 2. The results of the molecular dynamics simulation for the neuraminidase molecule with and without binding to berberine. A: Root mean square deviation (RMSD); B: Radius of Gyration (Rg); C: Total binding Energy (TE); D: Root-mean-square fluctuation (RMSF).

Alkaloids produce an anti-influenza effect by inhibiting neuraminidase and preventing the integration of the viral envelope and cell membrane (10). In one study to identify new compounds with plant neuraminidase inhibitory effects, Kim et al investigated the inhibitory activity of *Corydalis turtschaninovii* rhizome extract alkaloids against this viral enzyme. Among the compounds identified in that study, berberine exhibited strong inhibitory effects against viral neuraminidase (11). A study addressed the antiviral effects of berberine on the influenza virus and the

possible inhibitory effects of viral neuraminidase by this compound *in vitro*. Berberine could reduce the expression of viral neuraminidase at concentrations 0.00625-0.05 g/L (19). Specifically, synthetic compounds derived from berberine have been reported to produce potent inhibitory effects in inhibiting viral neuraminidase (20).

Many *in silico* molecular dynamics studies have been performed on many vital enzymes and proteins to predict the inhibitory or activating effects of various compounds on these enzymes and proteins. Liu et al conducted an *in silico* study on the effect of two inhibitors, zanamivir and oseltamivir, on neuraminidase and observed the inhibitory effects of these two compounds in addition to introducing the binding site of these two inhibitors in the binding site of the enzyme and the number of amino acids that are in the binding site with these inhibitors (21). The molecular dynamics results from this study also confirm that the binding of berberine to neuraminidase causes changes in its structure, possibly affecting the function and activity of the enzyme. Due to the binding of berberine to neuraminidase, a sharp decrease was observed in fluctuations in amino acids 374-379 and a sharp increase in fluctuations in amino acids 352-358, which can affect the enzyme's function. The neuraminidase protein consists of four identical polypeptides and accounts for approximately 10-20% of the total glycoproteins of the virion surface.

Four monomers are divided into four separate structural parts: consisting of the cytoplasmic tail of amino acids 1-6, the transmembrane region of amino acids 7-27, the stalk, and the catalytic head of amino acids 28-454 (4). The changes caused by the fluctuation of amino acids of neuraminidase due to binding to berberine are mostly related to the catalytic head part of the enzyme. Also, the direct binding of berberine to the active site of neuraminidase leads to a catalytic dysfunction of the enzyme, and the changes in the structure and the number of fluctuations in the active site of the enzyme caused by binding to berberine can indicate some dysfunction of the enzyme.

Conclusion

This study clearly showed the effects of docking and molecular dynamics of berberine on influenza virus neuraminidase. Berberine produces the possible effect and inhibits viral neuraminidase. Therefore, it seems that the design of drugs with similar characteristics to this compound can be effective in the treatment of influenza.

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Authors' contributions

Conception: MAS and MTM; Design: DA and JSC; Supervision: MTM; Data collection and/or processing: JSC and AHJ; Analysis and/or interpretation: JSC and AHJ; Writing: MAS, DA, AHJ, JSC, and MTM; Review and final revision approval: All authors.

Conflict of interests

None to declare.

Ethical considerations

The protocol of this study was approved by the Ethical Committee of Shahrekord University of Medical Sciences (ethics code: IR.SKUMS.REC.1397.235).

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