Archives of Razi Institute, Vol. 77, No. 6 (2022) 2181-2186

DOI: 10.22092/ARI.2022.358070.2145

Original Article

A Non-Structural Protein of Severe Acute Respiratory Syndrome Coronavirus 2 with a Potential Ability to Reduce Blood Glucose for Use in Controlling Type 2 Diabetes Mellitus

Danaeifar, M[1] *

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Received 8 March 2022; Accepted 7 April 2022
Corresponding Author: mdanaeifar@alumni.ut.ac.ir

Abstract

It is estimated that more than 400 million people worldwide are suffering from diabetes. There are two types of diabetes. Type 1 diabetes is the result of insufficient insulin secretion into the bloodstream, most often due to an autoimmune attack on the pancreas glands. Type 2 diabetes is caused by the inability of the surface ligands to adsorb the insulin from the bloodstream. The conventional medicines for diabetes mellitus include sulfonylureas, biguanide, thiazolidinediones, alpha-glucosidase inhibitors, and meglitinide. By February 2022, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had infected more than 391 million people worldwide, claiming 5.7 million lives and imposing heavy costs on the healthcare system. The present study aimed to assess the potential use of this non-structural SARS-CoV-2 protein in the treatment of type 2 diabetes mellitus. The nsp10 was structurally aligned with GoDrugBank therapeutic agents, and lixisenatide was found to have the most similar chemical structure. This drug is a glucagon-like peptide-1 (GLP1) receptor agonist used for the treatment of type 2 diabetes mellitus. The best molecular docking energy score for these two proteins was -301.47, and the ligand root mean square deviation was calculated to be 107.93 Å. The molecular dynamics for the stability of the nsp10 and GLP1R binding in triplicate for 150 ns demonstrated that the nsp10-GLP1R remained bound for more than 80 ns. This study indicated that the nsp10 protein can be further studied to be used as an antidiabetic medication.

Keywords: diabetes mellitus, nsp10, protein docking, SARS-CoV-2

1. Introduction

It is estimated that over 400 million people are suffering from diabetes around the world (1). One major factor contributing to the increased prevalence of diabetes is an elevated rate of obesity, and this condition is now the most serious physiologic pandemic disease (2). Diabetes is a disease in which blood sugar levels are elevated. This condition is one of the diseases characterized by hyperglycemia resulting from defects in the secretion of insulin or its effect on the target ligands. Numerous pathological conditions can lead to diabetes mellitus (DM) from autoimmune-mediated destruction of the pancreatic beta cells to disorders that result in resistance to insulin action (3).

Diabetes is manifested by various symptoms, such as weight loss, polyphagia, polyuria, blurred vision, and polydipsia (4). In the chronic stage of the disease, some other symptoms may appear, such as hyperglycemia with ketoacidosis or nonketotic hyperosmolar syndrome, neuropathy leading to renal failure, potential vision loss, foot ulcers due to the peripheral neuropathy, amputations, sexual dysfunction, and cardiovascular symptoms (5). There are two types of diabetes. Type 1 diabetes is the result of insufficient

secretion of insulin in the bloodstream which is generally due to the autoimmune attack on the pancreatic glands (6). Type 2 diabetes is caused by the inability of surface ligands to adsorb insulin from the bloodstream. Although the primary treatment for DM is lifestyle and diet change, drug administration is necessary for advanced stages of the disease (7). Conventional medicines for DM include sulfonylureas, biguanide, thiazolidinediones, alpha-glucosidase inhibitors, and meglitinide (8).

By February 2022, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had infected more than 391 million people worldwide, claiming 5.7 million lives and imposing heavy costs on the healthcare system (9). The SARS-CoV-2 is a member of the family of enveloped positive-sense single-stranded RNA viruses. It has been reported that this virus probably originated in bats (10). The examination of the SARS-CoV-2 genome revealed that there are 15 non-structural proteins, called nsp1 to nsp16, and four structural proteins (nucleocapsid (N), spike (S), membrane (M), and envelope (E)). Many nsps are involved in essential virus functions, for example, SARS-CoV-2 nsp12, along with nsp7 and nsp8, forms a multi-protein complex involved in RNA replication (11). The nsp10 which is a LIM type protein containing two zinc-finger motifs is one of these proteins structurally characterized by Rogstam, Nyblom (12). In light of the aforementioned issues, the present study aimed to evaluate the potential of this non-structural protein of SARS-CoV-2 in the treatment of type 2 diabetes mellitus.

2. Materials and Methods

2.1. Protein Sequence and Structure

The sequence and structure of the nsp10 protein were retrieved from the PDB database (PDB ID: 6ZCT).

2.2. Structural Homology Search

In order to find drugs with a similar structure to nsp10, its PDB was structurally aligned with all drug structures in the GoDrugBank database (13). The similarity search was set against the whole structure, and the threshold ST was obtained at 0.9.

2.3. ADME Tests

In order to predict the toxicity of the proteins on different body organs and tissues, eMolTox online webserver was used. The ADME@NCATS web server was also used to predict rat liver microsomal (RLM) stability, parallel artificial membrane permeability assay (PAMPA), solubility, human liver cytosolic (HLC) stability, and CYP450 toxicity tests of nsp10.

2.4. Target Protein Identification

The structurally similar drug was evaluated for its function and receptors in GoDrugBank, and the drug-receptor sequence was retrieved from the UniProt databank.

2.5. Protein-protein Docking

Molecular docking was performed between the nsp10 and the target receptor to study their interaction and the binding strength using the HDOCK web server (14).

2.6. Molecular Dynamic

Classical molecular dynamics simulations with SARS-CoV-2 nsp10 protein bound to the glucagon-like peptide-1 receptor (GLP1R) were performed using GROMACS 2018 software. The initial native structure of the SARS-CoV-2 nsp10 protein was retrieved from the RCSB Protein Data Bank (PDB ID: 6ZCT). The native structure of the glucagon-like peptide-1 receptor (GLP1R) was also obtained from the UniProt database (P43220). The water solvated forms of both proteins were obtained using TIP4P as the water model and within the rhombic dodecahedron box, and the minimum distance was set at 3.0 nm between protein atoms and the box. Contact analysis and the root mean square fluctuation (RMSF) were determined for each complex. The constants for the pressure and temperature were set at 1.01325 and 300k, respectively. In order to monitor the stability of the nsp10 in their native motion, the root mean square deviation (RMSD) was estimated.

2.7. Natural Product Likeness Test

In order to examine the potential of this protein to be

counted as a natural product drug, the PDB file format of the nsp10 was converted to Mol file type, and the resulting data were uploaded in the Natural Product Likeness Score calculator (NaPLeS).

3. Results

3.1. Structural Similarity Search

The general structure of the nsp10 is displayed in figure 1. The nsp10 was structurally aligned with GoDrugBank, and lixisenatide had the most similar chemical structure. This drug is a peptide composed of 44 amino acids and has an amide group at its C-terminus. It is a glucagon-like peptide-1 receptor (GLP-1R) agonist used to treat type 2 diabetes mellitus (T2DM). The GLP1R is a receptor protein found on the pancreatic beta cells and brain neurons. The GLP1R has 463 amino-acid residues and its tertiary structure consists of 15 helixes, 8 beta-strands, and 6 turns (Figure 1).

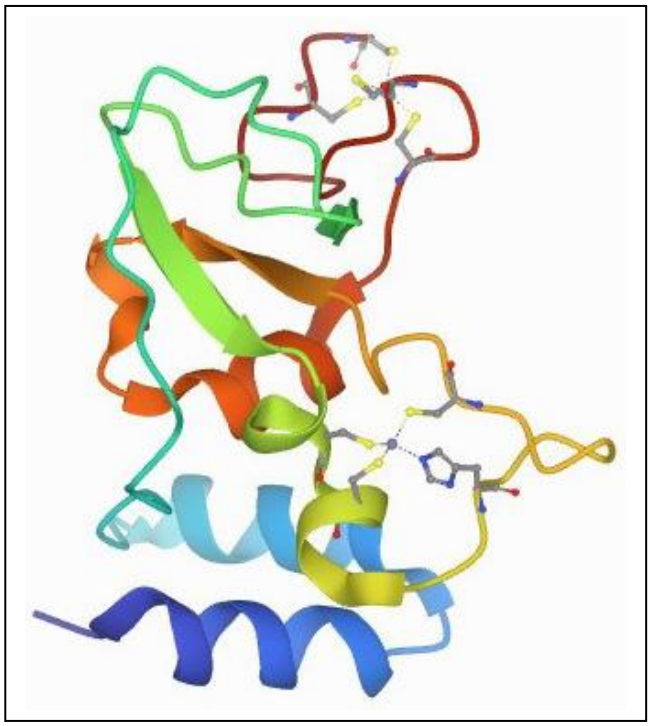

Figure 1. Tertiary structure of nsp10 protein (The simulation was carried on the solvated form of the protein).

3.2. Protein-Protein Docking

Protein-protein docking was performed using the HDOCK web server. This server uses a hybrid algorithm for the prediction and affinity of the binding based on both template-based and template-free docking, leading to the accuracy of the prediction (14). The best molecular docking energy score for these two proteins was -301.47, and the ligand rmsd (Å) was calculated to be 107.93 (named model 1). The binding site for the nsp10 was located at a region between amino acids 70 to 93, and for the GLP1R, it was located at amino acids 120 to 145. This region is the binding site of the receptor which can be targeted by the medications. The most prominent amino acids in the binding region of nsp10 were histidine and lysine, respectively. The binding schematic of model 1 and other models of ligand-receptor interaction are illustrated in figure 2, and their affinity is presented in table 1.

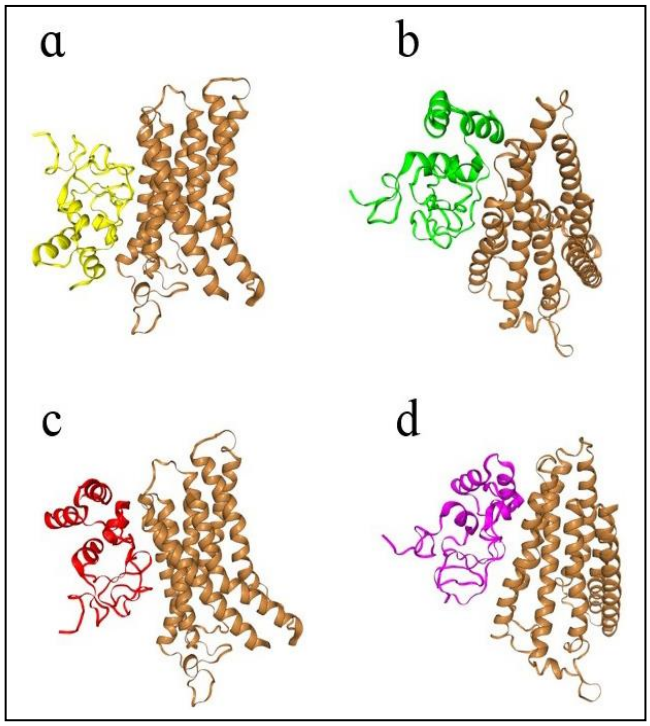

Figure 2. Four models of nsp10 protein of SARS-CoV-2 and glucagon-like peptide-1 receptor interactions. ɑ) Model 1 of interaction that had the best docking score. b, c, and d) Model 2, 3, and 4 of ligand-receptor interactions

Table 1. Docking score and rmsd quantities of various nsp10 and GLP1R interactions

Model	1	2	3	4
Docking Score	-301.47	-298.22	-292.54	-291.37
Ligand rmsd (A)	107.93	108.97	99.98	111.78

3.3. Stability of the Nsp10 and Glucagon-Like Peptide-1 Receptor Binding

The molecular dynamics technique was used to assess the stability of the nsp10 and the GLP1R binding in triplicate for 150 ns. The results demonstrated that the nsp10-GLPIR remained bound for more than 80 ns. The analysis of the RMSD of the protein backbone revealed that equilibrium was reached after 60 ns. The RMSF analysis was performed to study the protein backbone flexibility. The most significant flexibility was observed at residues 120-141 of nsp10.

3.4. ADM Simulations

The blood-brain barrier (BBB) test pointed out that nsp10 cannot be transmitted across the BBB and is therefore safe for brain cells. Rat liver microsomal stability module showed that nsp10 is stable with the predicted class (probability) of 0.93. The PAMPA test measure was predicted to be moderate or high permeability at pH 5, while at pH 7.4, it was low or moderate in the predicted class of 1. The results also indicated that it is highly soluble in the body fluids (predicted class of 0.99). The human liver cytosolic stability test demonstrated that this protein is very stable in the cytosol with a predicted class of 0.56. The CYP2C9-inhibitor and substrate tests were 0.53 and 0.56, respectively. For CYP2D6 inhibitor and substrate, it was calculated to be 0.55 and 0.53, while for CYP3A4 inhibitor and substrate, these values were 0.53 and 0.5. The probable toxicity of the nsp10 for different organs of the body is presented in table 2.

Table 2. Possible toxicity of the nsp10 protein of SARS-CoV-2 for various tissues of the body

Target	Action	Organ	Confidence
Hepatotoxicity	Activators of the heat shock response signaling pathway	Liver	0.994
Nephrotoxicity	Cytotoxicity in HEK293 cells - 8 hour	Kidney	0.99
Cardiotoxicity	Cytotoxicity in HEK293 cells - 16 hour	Heart	0.988
Neurotoxicity	Agonist of the androgen receptor (AR) signaling pathway	Central nervous system	0.99
Reproduction toxicity	Agonist of the androgen receptor (AR) signaling pathway	Endocrine	0.99
Cell toxicity	Agonist of H2AX	DNA Damage	0.992

4. Discussion

Changes in human lifestyle, habits, and diet, as well as emotional problems, have led to an increased prevalence of health conditions, such as obesity and diabetes (15). Due to the mentioned parameters, it is not impossible if we think that the progression pace of DM may exceed the current advances and treatments in this field. The SARS-CoV-2 has affected millions of people around the world, causing great fear and panic worldwide; nonetheless, this dangerous little creature can benefit us in some ways. The present study

demonstrated that the nsp10 non-structural protein of coronavirus has a considerable binding affinity to GLP1R, and it can be used as an agonist drug for the treatment of DM.

The toxicity tests pointed out that this protein has no significant off-target effects and cannot be transmitted through BBB, making it safe for the brain tissue. Since 2019, many researchers have studied the various proteins and ORFs of the SARS-CoV-2, and most of these studies focused on determining the pathogenesis mechanism of this virus (16). For instance, the ORF3a protein of the virus has been found to induce apoptosis in host cells (17). Many other studies have turned their attention to the spike protein, which mediates the virus binding and entry into the host cells by binding to the angiotensin-converting enzyme 2 (ACE2) (18). The nsp10 protein has been evaluated in several publications for its suitability to be used for antiviral drug development (19). In another study, the screening against nsp14/nsp10 exoribonucleases was performed to identify SARS-CoV-2 antiviral compounds (20).

In the same context, Mohammad, Alshawaf (21) conducted a study to assess the potential of natural products to abrogate the formation of the nsp10-nsp16 complex. They used the silico approach and observed that genkwanin-6-C-beta-glucopyranoside can tightly bind to the nsp10, thereby preventing it from making a complex with nsp16 that is essential for virus propagation. The covid-19 pandemic has negatively affected people across the globe; therefore, it is necessary to find effective measures to combat this challenge. The present research demonstrated that nsp10 protein can be further studied to be used as an antidiabetic drug, and maybe there are more proteins synthesized by SARS-CoV-2 that can be used as beneficial bioactive compounds.

Authors' Contribution

M. D. developed the original idea and performed the analyses. The acquisition of the data, the manuscript preparation, revision, and supervision were also carried out by M. D..

Conflict of Interest

The author declares that there is no potential conflict of interest related to this research and publication. I hereby confirm that I have reviewed and complied with the relevant Instructions to Authors, the Ethics in Publishing policy, and Conflicts of Interest disclosure.

References

1. Rajput DS, Basha SM, Xin Q, Gadekallu TR, Kaluri R, Lakshmanna K, et al. Providing diagnosis on diabetes using cloud computing environment to the people living in rural areas of India. J Ambient Intell Humaniz Comput. 2021:1-12.
2. Smyth S, Heron A. Diabetes and obesity: the twin epidemics. Nat Med. 2006;12(1):75-80.
3. Zimmet PZ, Magliano DJ, Herman WH, Shaw JE. Diabetes: a 21st century challenge. Lancet Diabetes & endocrinology. 2014;2(1):56-64.
4. Tao Z, Shi A, Zhao J. Epidemiological perspectives of diabetes. Cell Biochem Biophys. 2015;73(1):181-5.
5. Alam U, Asghar O, Azmi S, Malik RA. General aspects of diabetes mellitus. Handb Clin Neurol. 2014;126:211-22.
6. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, et al. Type 1 diabetes mellitus. Nat Rev Dis Primers. 2017;3(1):1-17.
7. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman medical journal. 2012;27(4):269.
8. Mullan RJ, Montori VM, Shah ND, Christianson TJ, Bryant SC, Guyatt GH, et al. The diabetes mellitus medication choice decision aid: a randomized trial. Arch Intern Med. 2009;169(17):1560-8.
9. Hopkins J. Coronavirus Resource Center 2022 [Available from: https://coronavirus.jhu.edu/map.html.
10. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022;23(1):3-20.
11. Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, et al. Structural insights into SARS-CoV-2 proteins. J Mol Biol. 2021;433(2):166725.

12. Rogstam A, Nyblom M, Christensen S, Sele C, Talibov VO, Lindvall T, et al. Crystal structure of non-structural protein 10 from severe acute respiratory syndrome coronavirus-2. Int J Mol Sci. 2020;21(19):7375.
13. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2018;46(1):1074-82.
14. Yan Y, Tao H, He J, Huang S-Y. The HDOCK server for integrated protein–protein docking. Nat Protoc. 2020;15(5):1829-52.
15. Myers J, Atwood JE, Froelicher V. Active lifestyle and diabetes. Am Heart Assoc; 2003. p. 2392-4.
16. Kim D, Lee J-Y, Yang J-S, Kim JW, Kim VN, Chang H. The architecture of SARS-CoV-2 transcriptome. Cell. 2020;181(4):914-21.
17. Ren Y, Shu T, Wu D, Mu J, Wang C, Huang M, et al. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. Cell Mol Immunol. 2020;17(8):881-3.
18. Hulswit R, De Haan C, Bosch B-J. Coronavirus spike protein and tropism changes. Adv Virus Res. 2016;96:29-57.
19. Lin S, Chen H, Ye F, Chen Z, Yang F, Zheng Y, et al. Crystal structure of SARS-CoV-2 nsp10/nsp16 2′-O-methylase and its implication on antiviral drug design. Signal Transduct Target Ther. 2020;5(1):1-4.
20. Canal B, McClure AW, Curran JF, Wu M, Ulferts R, Weissmann F, et al. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of Nsp14/nsp10 exoribonuclease. Biochem J. 2021;478(13):2445-64.
21. Mohammad A, Alshawaf E, Marafie SK, Abu-Farha M, Al-Mulla F, Abubaker J. Molecular simulation-based investigation of highly potent natural products to abrogate formation of the nsp10–nsp16 complex of sars-cov-2. Biomolecules. 2021;11(4):573.