Penta-peptide ATN-161 based neutralization mechanism of SARS-CoV-2 spike protein

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A R T I C L E   I N F O

Keywords:
ATN-161
ACE2
Protein-protein interactions
Severe acute respiratory syndrome coronavirus-2
Western blot

A B S T R A C T

SARS-CoV-2 has become a big challenge for the scientific community worldwide. SARS-CoV-2 enters into the host cell by the spike protein binding with an ACE2 receptor present on the host cell. Developing safe and effective inhibitor appears an urgent need to interrupt the binding of SARS-CoV-2 spike protein with ACE2 receptor in order to reduce the SARS-CoV-2 infection. We have examined the penta-peptide ATN-161 as potential inhibitor of ACE2 and SARS-CoV-2 spike protein binding, where ATN-161 has been commercially approved for safety and possess high affinity and specificity towards the receptor binding domain (RBD) of S1 subunit in SARS-CoV-2 spike protein. We carried out experiments and confirmed these phenomena that the virus bindings were indeed minimized. ATN-161 peptide can be used as an inhibitor of protein-protein interaction (PPI) stands as a crucial interaction in biological systems. The molecular docking finding suggests that the binding energy of the ACE2-spike protein complex is reduced in the presence of ATN-161. Protein-protein docking binding energy (-40.50 kcal/mol) of the spike glycoprotein toward the human ACE2 and binding of ATN-161 at their binding interface reduced the binding energy (-26.25 kcal/mol). The finding of this study suggests that ATN-161 peptide can mask the RBD of the spike protein and be considered as a neutralizing candidate by binding with the ACE2 receptor. Peptide-based masking of spike S1 protein (RBD) and its neutralization is a highly promising strategy to prevent virus penetration into the host cell. Thus masking of the RBD leads to the loss of receptor recognition property which can reduce the chance of infection host cells.

1. Introduction

The high amino acid sequence and structural similarity between the SARS-CoV-1 and SARS-CoV-2 spike proteins suggest that they belong to the same lineage [1]. The interaction between the SARS-CoV-2 surface spike protein (S1) and its receptor angiotensin-converting enzyme-2 (ACE2) serves in the penetration and replication of the virus particle into host cells and its replication [2]. Spike glycoprotein is a trimeric transmembrane protein that adopts multiple-folded conformation and each monomer comprises two functional S1 and S2 subunits [3]. The S1 subunit contains a receptor binding domain (RBD) that is involved in the binding to the host cell receptor [4]. However, the S2 subunit is involved in the fusion of the viral particle and host cell membrane [5]. ACE2 is membrane associated aminopeptidase and ubiquitously expressed in the lung, kidney, heart, gut, testis, blood vessels and brain [6]. ACE2 gives protection against organ damage in hypertension and cardiovascular diseases by counter-regulating the rennin-angiotensin system (RAS) [7]. The initial entry step of the SARS-CoV-2 S1 antigen is mediated by the binding of its cellular receptor ACE2 [8]. Researchers developing therapeutic inhibitor molecules that bind to the SARS-CoV-2 surface spike protein (S1) and combat the current pandemic situation [9]. SARS-CoV-2 harbors an Arg-Gly-Asp (RGD) motif and shows that the involvement of integrin facilitates the virus invasion into host cells [10]. Recently it was reported that RBD-ACE2 interfaces and identified key

Abbreviations: ACE2, Angiotensin-converting-enzyme 2; MERS-CoV, Middle East respiratory syndrome coronavirus; PBS, Phosphate buffer saline; PPI, Protein-protein interaction; PVDF, Polyvinylidene difluoride; RBD, receptor binding domain; RBM, receptor binding motif; SARS-CoV-1, severe acute respiratory syndrome coronavirus 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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https://doi.org/10.1016/j.bbrep.2021.101170
Received 7 June 2021; Received in revised form 25 October 2021; Accepted 8 November 2021
Available online 10 November 2021
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tolerated [13]. Antiviral peptides usually grab the shallow surface residues that are involved in contributing the binding strength of RBD-ACE2 [11]. Hsiang et al. reported that disruption of the SARS-CoV-2 S protein-ACE2 interaction by small peptides using biotinylated enzyme-linked immunosorbent assay (ELISA) [12]. In the current study, we adopted another direct approach using the Western blot procedure and demonstrated that SARS-CoV-2 S1 spike proteins are completely neutralized and do not show any sign of binding with the SARS-CoV-2 S1 antibody. Hence, we carried out a computational study and predicted interactions between SARS-CoV-2 spike glycoproteins and ACE2 receptor.

2. Neutralization of SARS-CoV-2 S1 by the peptide ATN-161

Peptide-based inhibitors (small molecules) could be an attractive alternative to control and block the antigen-binding site because they are more efficient and specific than other drugs and thus are better tolerated [13]. Antiviral peptides usually grab the shallow surface structures of the viral particle that are essential for its viral penetration into the host cell [14]. For instance, they may target viral proteins and interfere with their interactions, modulate conformational changes, alter the enzymatic activity, or control the host proteins that are essential for virus entry into host cells [15]. Peptide-based therapeutics also is an effective alternative to small chemical entity drugs because peptide-based inhibitors have low toxicity and easy synthesis steps [16]. Peptide possesses many favorable features including higher selectivity and potency, which make them effective at very low dose concentrations [17]. RBD targeting peptide of S1 subdomain interfere with ACE2 in the receptor-ligand interaction that is essential for the viral entry into the host cell. The initial and critical route of entry of both SARS-CoV-1 and SARS-CoV-2 viruses is the interaction between the viral S protein and ACE2 receptor. Therefore, impairing S-RBD binding to ACE2 has the potential to inhibit viral entry into human cells, presenting an opportunity for therapeutic intervention as a complement to vaccination strategies.

ATN-161 (Ac-Pro-His-Ser-Cys-Asn-NH2) is a small cysteine containing penta-peptide that was previously shown to inhibit prostate/breast cancer progression and metastasis in vivo [18]. The terminal side of the ATN-161 (PHSCN) peptide capped by acetylation and amidation has been shown to enhance the stability and bioactivity 30 folds [19]. It is derived from the synergy position of the fibronectin and interacts with integrin α5β1 [20]. Integrin α5β1 is the primary fibronectin (FN) receptor [21], responsible for cell migration and adhesion. Integrin α5β1-FN interactions are of particular interest as both proteins are ubiquitously expressed in various cell and tissue types to maintain the communication between cells and the extracellular matrix (ECM) [22]. Furthermore, α5β1 represents an integrin prototype that is fundamental to cellular processes, yet the conformational change through its molecular interaction with FN, a major component of the ECM [23]. ATN-161 binding to the Arg-Gly-Asp (RGD) binding pocket, and acts as an inhibitor of integrin α5β1 [18]. SARS-CoV-2 S1 spike protein binds to α5β1 and α5β1/hACE2 and consequently, the ACE2 binding is disrupted by non-RGD in the Vero-E6 (African green monkey Cercopithecus aethiops kidney epithelial cells, ATCC-CRL 1586) cell lines. In this study, it was found that the interaction of ATN-161 peptide reasonably antagonizes the ACE2-SARS-CoV-2 S1 RBD interaction.

3. Materials and sample preparation

ATN-161 peptide (SML2079) was acquired from Sigma-Aldrich. ATN-161 peptide 1.0 mg was dissolved in 1 mL 1X PBS and stored in -20 °C for further use in the experiment. Recombinant SARS-CoV-2 spike/S1 subunit-His tag (Cat # 40591-V08H) and MERS-CoV spike/S1

Table 1

A comparative summary of performed experiment Western blot of SARS-CoV-2 S1/MERS-CoV S1 antigen without and with increasing ATN-161 peptide ratio.

| S. No. | SARS-CoV-2 S1 | MERS-CoV S1 | Ratio | Band intensity |
|-------|--------------|-------------|-------|---------------|
| 1.    | ✓            | none        | 1:0   | ++++          |
| 2.    | ✓            | none        | 1:1   | ++++          |
| 3.    | ✓            | none        | 1:2   | ++++          |
| 4.    | ✓            | none        | 1:3   | ++++          |
| 5.    | ✓            | none        | 1:4   | ++++          |
| 6.    | ✓            | none        | 1:5   | + +          |
| 7.    | ✓            | none        | 1:10  | + +          |
| 8.    | ✓            | none        | 1:20  | + +          |
| 9.    | ✓            | none        | 1:50  | + +          |
| 10.   | ✓            | none        | 1:40  | Not detected |
| 11.   | ✓            | none        | 1:50  | No detection |
| 12.   | none         | ✓           | 1:2   | No detection |

Used symbol explanation:

- +: poor band detected.
- ++: band detected.
- +++: strong band detected.
- ++++: very strong band detected.
- Very strong band detected.
- +: very poor band detected.
- None: not detected.

Fig. 1. (A) Western blot of SARS-CoV-2 S1 antigen with increasing concentration of the ATN-161 peptide. Lane 1 is the experiment performed in the absence of the ATN-161 (only SARS-CoV-2 S1 antigen). Lane 12 is for control experiment MERS-CoV S1 antigen and in the presence of ATN-161 peptide.

(B) The obtained Western blot bands intensities were analyzed by ImageJ program. The data represent the average of three individual experiments.

Table 1

| S. No. | SARS-CoV-2 S1 | MERS-CoV S1 | Ratio | Band intensity |
|-------|--------------|-------------|-------|---------------|
| 1.    | ✓            | none        | 1:0   | ++++          |
| 2.    | ✓            | none        | 1:1   | ++++          |
| 3.    | ✓            | none        | 1:2   | ++++          |
| 4.    | ✓            | none        | 1:3   | ++++          |
| 5.    | ✓            | none        | 1:4   | ++++          |
| 6.    | ✓            | none        | 1:5   | + +          |
| 7.    | ✓            | none        | 1:10  | + +          |
| 8.    | ✓            | none        | 1:20  | + +          |
| 9.    | ✓            | none        | 1:50  | + +          |
| 10.   | ✓            | none        | 1:40  | Not detected |
| 11.   | ✓            | none        | 1:50  | No detection |
| 12.   | none         | ✓           | 1:2   | No detection |

Used symbol explanation:

- +: poor band detected.
- ++: band detected.
- +++: strong band detected.
- ++++: very strong band detected.
- Very strong band detected.
- +: very poor band detected.
- None: not detected.
subunit His tag amino acid 1–725 recombinant protein (Cat# 40069-V08H) were purchased from Sino Biological Inc, Beijing, China. SARS-CoV-2 S1 and MERS-CoV S1 antigen stock were prepared in 1X PBS buffer at pH 7.4, to prevent denaturation, stored at 4°C. Monoclonal rabbit anti-spike S1 (40150-R007) was purchased from Sino Biological Inc, Beijing, China. Secondary antibody goat pAb to rabbit IgG HRP-conjugated was purchased from Abcam (ab6721). PVDF transfer membrane (Immobilon P transfer membrane) was purchased from Merck Millipore Ltd, Korea. For the making of buffer and washing solutions deionized (DI) water 18.2 MΩ/cm, was obtained from an MDM Wellix Plus water purifier system (MDM Corporation, South Korea). Skim milk powder (SM2010) was used as a blocking reagent in Western blotting purchased from Georgiachem, South Korea. The other reagents used in this study were of analytical purity standard and used without any further purification.

4. Experiments

4.1. Western blot of SARS-CoV-2 surface spike glycoprotein S1

SARS-CoV-2 surface spike glycoprotein S1 antigens 1 μg/mL in the absence and presence of ATN-161 peptide were separated under reducing conditions of 12% polyacrylamide gels [24]. After electrophoresis, protein bands were transferred from the SDS-PAGE gel to the PVDF membrane. The membrane was blocked in 5% (w/v) skim milk prepared in 1X phosphate-buffered saline containing 0.05% Tween-20 (PBST) and left overnight at 4°C. After overnight incubation, the blocking solution was discarded and washed three times with 1X PBST. SARS-CoV-2 (2019-nCoV) spike protein S1 primary antibodies 1:5000 dilution (200 ng/mL) prepared in 5% (w/v) skim milk in 1X PBST. The PVDF membrane was soaked in diluted primary antibody containing blocking solution and left for 2 h at room temperature on a rotating shaker plate. After three washes with 1X PBST, the PVDF membrane was further soaked in 5% skim milk containing secondary antibody goat pAb to rabbit IgG HRP-conjugated (1:5000 dilution) and incubated for 60 min at room temperature on a rotating shaker plate. PVDF membrane was washed 5 times with 1X PBST to remove unbound remains of secondary antibody. ECL solution (A and B) was mixed in 1:1 ratio following the proportion of solution A and B provided by incubated for 3 min allow to complete the reaction. The generated signal was exposed in the darkroom with an exposure time of 40 s and the X-ray film developed by immersing in the appropriate developing and fixing solutions for 5 min each respectively. The band intensities were analyzed by ImageJ 1.45 software according to the manufacturer’s instructions.

4.2. In silico analysis

The 3D crystal structure of the SARS-CoV-2 spike protein binding domain complexed with ACE2 was retrieved from the protein data bank in order to perform protein-protein and protein-peptide interactions (PDB ID: 6LZG). It is a two-mer heterogeneous structure (chain A is ACE2 and B is spike protein S1) [25]. The 2D structure of ATN-161 was accessed from the PubChem database (CID: 9960285). UCSF Chimera was used to performing energy minimization and protein/ligand preparation on both protein and peptide structures [26]. The web version of PatchDock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) was used to perform protein-protein docking simulations between SARS-CoV-2 spike protein and ACE2 receptor, S1 protein and ATN-161 peptide, and ACE2 and complex of S1 protein-ATN-161, which were then refined and ranked with FireDock (http://bioinfo3d.cs.tau.ac.il/FireDock/). PatchDock generated 100 predictions for each interaction, which were then submitted to FireDock to identify the 10 best solutions based on global energy.

5. Results and discussion

5.1. Western blot studies for antigen-antibody interaction

Protein-protein interaction (PPI) stands as a crucial interaction in biological systems on which many scientific studies are focused.
Typically, proteins rarely do their functionality alone throughout in vivo conditions and therefore, they are usually dependent on their activities that can affect some other proteins. According to estimations, about 80% of proteins work in complexes [27]. These kinds of relationships can make some structural and functional changes in proteins that in many cases cause positive impacts and occur in normal biological functions while resulting in the inducement of certain diseases in some other cases [28]. In this study, we investigated the binding of the SARS-CoV-2 S1 antigen with the SARS-CoV-2 S1 antibody and inhibition of antigen-antibody binding by the presence of peptide ATN-161. To find out the S1 spike protein binding with antigen we performed the Western blot experiment in increasing the molar ratio of ATN-161 peptide in the eleven concentration of ATN-161 titration. The observed band of Western blot band intensity shows that the increasing concentration of ATN-161 exhibits the inhibition of antigen-antibody binding (Fig. 1). Furthermore, the application of ATN-161 reduced the binding of the SARS-CoV-2 S1 antigen to the SARS-CoV-2 S1 antibody. Table 1 describes the Western blot experimental conditions and uses SARS-CoV-2 S1 antigen and ATN-161 peptide ratio. The ratio 1:40 and 1:50 (SARS-CoV-2 S1 antigen:ATN161) is not showing any trace of binding of SARS-CoV-2 S1 and SARS-CoV-2 S1 antibody that indicates the blockage of the antibody binding sites by ATN-161 peptide. Lane 12 shows the results of 1:2 (MERS-CoV-2 S1 antigen:ATN-161) molar ratio do not show any trace in the Western blot. Band intensities were analyzed by

Fig. 3. (A)_Structure of SARS-CoV-2 S1 glycoprotein bound to its receptor ACE2. The S1 glycoprotein shown in light green, and its receptor ACE2 is shown in magenta color. Close up view of interacting interface between RBD and ACE2 (B) The docked structure of SARS-CoV-2 S1 glycoprotein bound to its receptor ACE2 and ATN-161 (shown in yellow ball). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Table 2
Interacting amino acid residues between ACE2 and SARS-CoV-2 spike protein. Chain A: ACE2 and Chain B: Spike protein.

| S. No. | Distance (Å) | Bond nature | Types From | To |
|--------|--------------|-------------|------------|----|
| 1.     | 3.09         | Electrostatic | Salt bridge | B: Lys417: NZ A: Glu40: OE1 |
| 2.     | 4.51         | Electrostatic | Attractive charge | B: Arg403: NH1 A: Glu50: OE2 |
| 3.     | 5.52         | Electrostatic | Attractive charge | B: Arg403: NH2 A: Glu45: OE2 |
| 4.     | 3.04         | Hydrogen bond | Conventional hydrogen bond | A: Asn137: ND2 B: Arg457: O |
| 5.     | 3.33         | Hydrogen bond | Conventional hydrogen bond | A: Asn137: ND2 B: Lys458: O |
| 6.     | 3.37         | Hydrogen bond | Conventional hydrogen bond | A: Ser280: OG B: Tyr449: OH |
| 7.     | 2.89         | Hydrogen bond | Conventional hydrogen bond | A: Lys441: NZ B: Thr500: OG1 |
| 8.     | 2.78         | Hydrogen bond | Conventional hydrogen bond | A: Tyr453: OH B: Glu150: OE1 |
| 9.     | 3.30         | Hydrogen bond | Conventional hydrogen bond | A: Arg457:N B: Glu40: OE1 |
| 10.    | 3.02         | Hydrogen bond | Carbon hydrogen bond | A: Ser280: CB B: Gly446: O |
| 11.    | 3.07         | Hydrogen bond | Carbon hydrogen bond | A: Phe456: CA B: Glu40: OE1 |
| 12.    | 5.47         | Hydrophobic | Alkyl | A: Pro289: B: Val445 |
| 13.    | 4.26         | Hydrophobic | Alkyl | A: Lys456: B: Val503 |
| 14.    | 3.04         | Hydrophobic | Alkyl | A: Ala475: B: Pro135 |
| 15.    | 5.33         | Hydrophobic | π-Alkyl | A: Tyr449: B: Leu156 |

Table 3
Interacting amino acid residues between ACE2 with spike protein and ATN-161 complex (SARS-CoV-2 S + ATN-161). Chain A: ACE2 and Chain B: Spike protein.

| S. No. | Distance (Å) | Bond nature | Types From | To |
|--------|--------------|-------------|------------|----|
| 1.     | 5.59         | Electrostatic | Attractive charge | B: Arg346: NH1 A: Asp494: OD1 |
| 2.     | 4.07         | Electrostatic | Attractive charge | B: Lys462: NZ A: Asp136: OD2 |
| 3.     | 4.57         | Electrostatic | Attractive charge | B: Arg466: NH1 A: Glu171: OE2 |
| 4.     | 3.25         | Hydrogen bond | Conventional hydrogen bond | A: Gln139: NE2 B: Glu516: OE1 |
| 5.     | 3.08         | Hydrogen bond | Conventional hydrogen bond | A: Tyr158: OH B: Thr470: OG1 |
| 6.     | 2.68         | Hydrogen bond | Conventional hydrogen bond | A: Lys174: NZ B: Asn354: OD1 |
| 7.     | 3.59         | Hydrogen bond | Carbon hydrogen bond | A: Pro492: CD B: Arg346: CD |
| 8.     | 2.89         | Hydrogen bond | Carbon hydrogen bond | A: Ala614: CB B: Phe490 |
| 9.     | 2.55         | Hydrophobic | σπ | A: Ala162: B: Ile468 |
| 10.    | 4.04         | Hydrophobic | Alkyl | A: Leu162 B: Ile468 |
| 11.    | 5.28         | Hydrophobic | π-alkyl | A: Tyr996 B: Pro138 |
| 12.    | 5.28         | Hydrophobic | π-alkyl | A: Tyr996 B: Pro138 |

5.2. Molecular docking between spike protein fragment and human ACE2 receptor

Molecular docking is a powerful tool that can be used to dock the binding of a peptide or ligand at the preferred site and orientation on a macromolecule [29-31]. The protein-protein interaction energy between spike protein and its receptor ACE2 is an important criterion to evaluate the effect of peptide, which could bind to the binding surface between spike protein and ACE2 receptor. RBD, located in the S1 subunit and binds to the host cell ACE2 receptor, while S2 functions in the membrane fusion during the infection process [25]. The RBD of SARS-CoV-2 and SARS-CoV RBD have an identical 3-D structure in the receptor ACE2 binding domain that maintains van der Waals forces [32]. The presence of a furin-like cleavage site at the S1/S2 boundary results in the cleavage of SARS-CoV-2 S glycoprotein before viral penetration made into the host cell. The inhibition of the furin-cleavage site could be a potential therapeutic target against SARS-CoV-2 infection [33,34].

Binding affinity is defined as the strength of protein-protein interaction, which is related to the cellular functions of those proteins. This binding energy can be expressed as a physicochemical parameter. Protein-protein interaction (PPi) of ACE2 and SARS-CoV-2 S (ligand-receptor), detailed structural insights are shown in Fig. 3A. Human ACE2 receptor (PDB: 6Z2G) is considered as receptor protein for molecular docking study of S1 spike protein (RBD). The molecular docking results revealed that there were multiple intermolecular attractions between the RBD of SARS-CoV-2 S1 and the ACE2 receptor. Docking structure of A chain of human ACE2 receptor binds with SARS-CoV-2 spike protein fragment with binding energy -40.50 kcal/mol. When ACE2 receptor binding peptides with the S1 protein fragment, a conformational change occurs after the ATN-161 binding (shown in Fig. 3A and B). Several amino acid positions of the interface between RBM and receptor ACE2 have been involved in binding through the formation of hydrogen bonds and salt bridges are shown in Fig. 3. Interacting amino acids of spike protein fragment Lys417, Arg403, Tyr453, Arg457, Phe456, Ala475, Tyr449, Arg457, Lys458, Tyr499, Thr500, Gly446, Val503 with amino acids present in the distorted site of ACE2 are Asn139, Ser280, Lys441, Ser280, Pro289, Lys363, Glu180, Glu145, Pro135, Leu156 (Table 2 and Fig. 3A). The amino acid sequence of RBD including the receptor binding motif (RBM) directly makes contact with ACE2. There are many critical amino acid residues of spike protein involved in binding with ACE2, particularly provide favorable interactions with human ACE2. Other critical residues in ACE2 particularly Asn139 present on its surface and strongly bind and make contact with RBM [35].

The docked poses are presented in Fig. 3B along with the residues of ImageJ software. The SARS-CoV-2 S1 antigen and SARS-CoV-2 S1 antibody binding decreased significantly treated with ATN161. The decreased band area also showed a dose-dependent trend in the high ATN-16 peptide concentration (Fig. 1B). In the figure, 2A it is depicted that entry step of the SARS-CoV-2 virus into the host cell receptor (ACE2) in the absence and presence of the ATN-161 peptide. In the absence of the ATN-161, the SARS-CoV-2 virus binds with its receptor because the RBD domain of the S1 subunit is masked by the peptide ATN-161. On the basis of findings, the ATN-161 is concluded to have masked the RBD of the SARS-CoV-2 virus present on the S1 subdomain. In the Fig. 2C it is depicted that experimental condition is shown for the Western blot of SARS-CoV-2 S1 antigen and SARS-CoV-2 S1 antibody binding in the absence and presence of ATN-161 peptide.
the protein with which the ATN-161 molecules interact. Hence, a critical analysis is necessary to assess the performance of the ATN-161 in binding to the SARS-CoV-2 S1 protein. Table 3 reports the detailed protein-protein (ACE2 and SARS-CoV-2 S1) interaction with the ligand ATN-161 molecules. The main interacting residues of the SARS-CoV-2 S1 are Arg346, Lys462, Arg466, Arg346, Tyr396, Gln516, Thr470, Asn354, Tyr351, Phe490 and Ile468 with ACE2 receptor. ATN-161 peptide makes hydrogen bonding as well as hydrophobic interactions with the spike amino acid residues. The spike S1 residues Glu516, Thr470, Asn354, and Tyr354 are involved in the direct hydrogen bonding with the ATN-161. The individual interactions of the ligand molecules with different residues of the RDB are also reported along with the total binding energy. The binding energy value predicted that the best ligand to interfere with the SARS-CoV-2 spike glycoprotein and thus can interfere with the attachment of SARS-CoV-2 with host cells inhibiting the very first stage of viral infection and limit the spread of SARS-CoV-2 infection.

6. Conclusion

The peptide based inhibitor ATN-161 inhibits the SARS-CoV-2 S1 antigen and SARS-CoV-2 S1 antibody binding by neutralizing the RBD of the spike protein. Thus, ATN-161 can be an alternative solution to neutralize the RBD-ACE2 interaction by appropriately covering the extended protein contact interface. ATN-161 inhibiting the binding of the RBD of SARS-CoV-2 spike glycoprotein with host receptor through molecular docking. The ATN-161 binds with SARS-CoV-2 spike protein and inhibits the recognition and binding property of its ACE2 receptors. In addition, the vaccines are still out of reach of many low-middle-income countries. Thus, the patients without the need for hospitalization mouth or nasal spray formulation of ATN-161 and its applications can be neutralizing the RBD of SARS-CoV-2 spike protein ATN-161 formulation can be a protective measure to limit the spread of SARS-CoV-2 infection. However, this proposal needs further clinical and experimental studies.

Authors credit

Gulam Rabbani: Designed the experiment plan, performed the experiment, data analysis, manuscript writing and editing.
Saeyoung Nate Ahn: Manuscript writing and editing.
Hyunhwa Kwon: Manuscript editing.
Khurshid Ahmad: Performed molecular docking and data analysis.
Inho Choi: Performed molecular docking and data analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this communication.

Acknowledgments

This study is supported by the internal fund of the Nano Diagnostics & Devices (NDD), Gumi-si, Gyeongbuk, 39253, Republic of Korea. The authors are thankful to Dr. Asrar Ahmad, Department of Physiology and Biophysics, Howard University College of Medicine, Washington, DC for the Western blot image analysis.

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