Rational Design of a Hybrid Peptide against Severe Acute Respiratory Syndrome Coronavirus 2 Using Melittin and Angiotensin-Converting Enzyme 2 as Pharmaceutical Agents

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Abstract

Background: Management of severe acute respiratory syndrome coronavirus 2 in humans depends on the availability of vaccines or effective drugs. Studies have shown that angiotensin-converting enzyme 2 (ACE2) is responsible for binding the viral spike glycoproteins to human cells. Melittin from the bee venom of Apis mellifera is a peptide with antimicrobial activities.

Materials and Methods: In this study, important amino acid residues of ACE2 interacting with spike glycoproteins of the virus were described based on the ACE2-spike–glycoprotein interface. This has been previously analyzed by Robson in crystal structures of the receptors and ligands. Flexible linkers and 31 amino acid residues from N-terminal of ACE2 as coronavirus spike binding domains (SBDs) were added to 17 N-terminal amino acids of melittin (the hydrophobic motif) to construct a hybrid peptide or M-ACE2SBD. Then, secondary and tertiary structures of the peptide were predicted.

Results: Docking of the hybrid peptide and coronavirus SBDs was carried out as well. Previous studies showed that toxicity and hemolytic activity of the melittin hydrophobic motif decreased in comparison to native melittin due to the lack of peptide binding to the exposed anionic lipids of the human cell membranes and hence the novel peptide can be recommended as an appropriate drug for clinical uses.

Conclusion: This study has hypothesized that 17 N-terminal amino acids of the mutant melittin used in M-ACE2SBD design are potentially hydrophobic and attached coronavirus-2 through lipid envelope of the virus.

Keywords: Angiotensin-Converting Enzyme 2, Covid-19, glycoproteins

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel strain of coronavirus that causes coronavirus disease 2019 (COVID-19), a respiratory infection with high mortality rates that was first detected in China in late 2019. Currently, new variants of the virus are evolving due to the virus multiple spike protein mutations. The infection has spread rapidly as a global risk. Management of COVID-19 human infection depends on the characteristics of the virus, including transmission capability, severity of resulting infection, and availability of vaccines and medicines to control severe side-effects of COVID-19. Coronaviruses are zoonotic agents, transmitting from animals to humans. However, the exact source of 2019-nCoV has not been identified. Evolution of 2019-nCoV may be resulted from RNA recombination in the virus. The most frequent breakpoint combination is located in the S gene of coronavirus, encoding spike glycoproteins.
Considering the broad prevalence and large genetic diversity of the current coronavirus, the virus’ close proximity and frequent recombination have been expected to emerge its novel variants. Based on the previous studies, angiotensin-converting enzyme type 2 (ACE2) that acts synergically with other receptors such as, transmembrane protease serine 2, neuropilin-1, and compact disc 147 is responsible for binding SARS-CoV-2 spike glycoproteins to epithelial cells of the respiratory and alimentary tracts. Once a human target protein and its relevant binding site on SARS-CoV-2 are clearly understood, the associated amino acids can be used for designing of potential therapeutic peptides. Therefore, modern computer-driven strategies and invention of automatatable peptide synthesis platforms can be helpful. Melittin from the bee venom of Apis mellifera is a peptide that shows antimicrobial, antitumor, and antiviral activities as well as other biological activities. The antimicrobial mechanism of melittin includes destruction of cell membranes. However, strong cytotoxicity of melittin limits its clinical uses.[5-7] In the current study, an artificial hybrid peptide was designed that may be used as a potential therapeutic peptide, consisting of 17 N-terminal residues of melittin with potential hydrophobic characteristics. Therefore, the peptide can attach the lipid envelope of SARS-CoV-2 with less toxicities for human cells, compared to native melittin. Another region of this hybrid peptide is associated to ACE2 domain, which is responsible for binding of SARS-CoV-2 spike glycoproteins to the respiratory and alimentary tract cells.

**Materials and Methods**

Peptides are generally interested as great candidates for medical therapies. The major sources of data on melittin, SARS-CoV-2 virus spike glycoproteins and human ACE2, include National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and Protein Data Bank (PDB: www.rcsb.org). Briefly, 6vw1 PDB entry was retrieved from PDB [Figure 1]. Three-dimensional (3D) crystal structures of the receptors and ligands, including ACE2 spike binding domain (SBD) and SARS-CoV-2 spike glycoprotein interface, have recently been described by Robson.[9] Associated amino acid residues in ACE2 that interact with the viral spike glycoproteins were investigated. Then, 17 N-terminal amino acids of melittin (the hydrophobic motif) and a flexible linker were added to 31 amino acid residues from the N-terminal of ACE2SBD to construct a hybrid peptide (M-ACE2SBD).[9]

Using online computer pl/Mw tool from Expasy (www.expasy.org), physicochemical characteristics of the designed peptide such as molecular weight and isoelectric point were predicted. The secondary and tertiary structures of the highlighted peptide were predicted using PEP-FOLD3 server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3). Docking of M-ACE2SBD and receptor-binding domain (RBD) coronavirus spike glycoproteins was carried out using online server of Jiangsu University of Technology, China (https://ncov.schanglab.org.cn) [Table 1].

**Results**

**Melittin hydrophobic motif**

Naturally, native melittin is consisted of 26 amino acids with a primary structure [Figure 2a]. In fact, 13 of the first 20 residues of this peptide are hydrophobic and only two are charged, whereas four of the last six residues are charged (amino acid sequences of lysin arginine lysin arginine [KRKR] and the remaining two are polar. The amino acid residues close to melittin N-and C-terminal regions are majorly hydrophobic and hydrophilic, respectively. Polar and nonpolar residues are roughly distributed symmetrically on the two sides of each helix, leading to the formation of an amphiphilic molecular configuration. After binding to lipid membranes, melittin presents a bent rod-like conformation with two α-helices, which are connected by a non-α-helical link, as demonstrated in previous studies by experiments and molecular dynamic simulations [Figure 2b].[8-11] The 17 N-terminal potentially hydrophobic residues of melittin include GIGAVLKV * TTGLPALI. The “L*” wild type has been replaced by “A” followed by a flexible peptide linker of alanine-proline-glycine (underlined) as GIGAVLKVATTGLPALIAPG.

**Angiotensin-converting enzyme 2 spike binding domains**

The interface of ACE2 that interacts with the virus spike glycoproteins is shown in Figure 1.

Amino acid residues in the rectangle belong to the bent α-helix of ACE2 (green) and interact with amino acid residues in

**Figure 1:** Structure of severe acute respiratory syndrome coronavirus 2 receptor-binding domain complexed with its receptor human angiotensin-converting enzyme 2 (Protein Data Bank: 6Vw1)

**Figure 2:** (a) Primary structure and (b) melittin hydrophobic motif structure. Blue, hydrophobic amino acids; orange, hydrophilic amino acids; and red, charged amino acids.
a stretched loop of SBD of SARS-CoV-2 (orange). These residues are located within a distance of 4 Å of each other and hence naturally counteract and are described as follows:

- STIEEQAKTFLDKFNHEAEDLFYQSSLASWN
- GFNCYFPLQSYGFQPT.

In this alignment, ACE2 helix includes 31 amino acid residues (green) and the extended chain of coronavirus spike glycoprotein-binding domain includes 16 amino acid residues (orange) with similar lengths in 3D views. Sequence from 17 N-terminal amino acids of melittin (L * wild type replaced by A) was followed by a flexible peptide linker of alanine-proline-glycine (underlined) with the remaining 31 residues belonged to ACE2SBD as GIGAVLKVFATTGLPAILAGPS TIEEQAKTFLDKFNHEAEDLFYQSSLASWN (M-ACE2SBD). Peptide folding prediction of the hybrid peptide and structural characterization of M-ACE2SBD were carried out using PEP-FOLD3 [Figure 3]. Furthermore, PI/Mw of M-ACE2SBD was calculated as PI = 4.64 and Mw = 5451 D using Expasy.

**Docking results**

The minimum binding energy scores of docking are shown in Table 1. Docking was carried out to identify minimum binding energy scores for the interactions of the highlighted sequences using the online webserver of Jiangsu University of Technology, China. The minimum binding energy for the interactions between M-ACE2SBD and coronavirus spike glycoprotein RBD was calculated as -276 kcal/mol. In general, M-ACE2SBD has been shown to include good binding scores for the coronavirus SBD and may serve as a therapeutic candidate for SARS-CoV-2.

**Discussion**

In this study, a potential α-helix upper domain of ACE2 was used as a virus-binding sequence for the construction of a hybrid peptide against SARS-CoV-2. Designing of hybrid peptides with SARS-CoV-2-binding domain of ACE2 and virus envelope disrupting motif of melittin as a therapeutic vehicle against the virus needs further attentions. Bioinformatics is a powerful tool for the study of protein sequences. In the present study, an artificial hybrid peptide of M-ACE2SBD with 51 amino acids was designed, including GIGAVLKVTGTGLPAILAGPS TIEEQAKTFLDKFNHEAEDLFYQSSLASWN. Sequence of 17 N-terminal amino acids belonged to melittin hydrophobic motif included GIGAVL * KVLTTGLPAl (L * wild type replaced by A to decrease hemolytic activity). According to Robson, sequence of ACE2SBD included STIEEQAKTFLDKFNHEAEDLFYQSSLASWN. This peptide was ligated using a flexible peptide linker of alanine-proline-glycine. Similar lengths were identified in the alignment of 31 amino acid residues from ACE2 helix with 16 amino acid residues from the stretched loop of the virus spike glycoproteins [Figure 1]. Each amino acid in α-helix of ACE2SBD increased the overall length by approximately 1.5 Å. However, each amino acid in β-strand or stretched chain of the virus spike glycoproteins that interacted with ACE2SBD increased the molecule length by approximately 3.5 Å. In general, associated geometry usually sets the two sequences as one-to-one spatial correspondence and facilitates their interactions close at the interface, causing overall intimate contacts. Conserved sequences or domains, which are recognizable in various coronavirus genomes, seem to include important functions. These sites may less likely acquire resistance by mutations. Conformational analysis of the spike glycoprotein binding to ACE2 suggests conservation of the glycoprotein and its appropriateness for vaccine design and therapeutic drug discovery. However, a limited number of accessible conserved sites exist in the spike glycoproteins. If amino acid residues of the stretched loop of the virus spike glycoproteins produce scape mutations, the designed peptides may not be helpful and drug resistance may appear. No significant information is available on the involvement of glycosylation in major interior interaction face of the complex and intimate interactions occurring between the relative amino acid residues. Modification of antimicrobial peptide structures fascinates researchers to design hybrid peptides that act further specifically and are safer for clinical uses. Studies have revealed that the leucine zipper motif of melittin plays critical

| Rank | Score value |
|------|-------------|
| 1    | -276.72     |
| 2    | -268.98     |
| 3    | -260.05     |
| 4    | -258.18     |
| 5    | -249.79     |
| 6    | -246.67     |
| 7    | -244.230    |
| 8    | -243.005    |
| 9    | -241.855    |
| 10   | -241.75     |
roles in the substance hemolytic activity.\textsuperscript{[12]} In the present study, 17 N-terminal residues of melittin were selected for the construction of M-ACE2SBD, lacking leucine 6 and tryptophan 19 of melittin. Blondelle showed that tryptophan 19 of melittin affected the hemolytic activity of this peptide.\textsuperscript{[13]} Thus, hemolytic activity of the designed peptide majorly decreased. Based on the previous studies, four basic amino acids (KRKR) at C-terminal of melittin are responsible for the peptide binding to exposed anionic lipids of the cell membranes.\textsuperscript{[9]} Therefore, lack of the four basic amino acids decreases melittin cytotoxicity. Studies have demonstrated that SARS-CoV-2 can effectively be inactivated by lipid solvents, including ether (75%) and ethanol.\textsuperscript{[14]} As previously described, only a small fraction of melittin is in a pore-competent transmembrane state at the same time and 17 N-terminal residues of melittin are potentially hydrophobic, which attach SARS-CoV-2 via lipid envelope of the virus. Naturally, melittin folds into an amphipathic $\alpha$-helix with a nonpolar surface that drives partitioning into bilayer lipid membranes. The cationic C-terminal segment of melittin drives its binding to anionic lipids; however, M-ACE2SBD lacks this segment. Melittin-$\alpha$-helix is separated into two structurally independent segments by a critical helix-breaking proline residue at position 14, resulting in a dynamic disordered pore state. In addition to these basic architectural principles, melittin is mostly monomeric in membranes, including its helical axis predominantly oriented parallel to the membrane surface.\textsuperscript{[15]} Based on the docking results, the minimum binding energy between M-ACE2SBD and the virus spike glycoproteins was $-276$ kcal/mol. Therefore, M-ACE2SBD may help researchers treat COVID-19. However, these data need further \textit{in vitro} and \textit{in vivo} assessments to verify M-ACE2SBD as a drug against COVID-19. Recent studies have shown that patients infected with SARS-CoV-2 suffer from pneumonia, mostly with severely low oxygen saturation levels of only 50%. Primarily, COVID-19 pneumonia causes oxygen deprivation that is difficult to detect because patients may not experience significant breathing difficulties, hence causing a condition termed silent hypoxia. Under this condition, blood oxygen level decreases, leading to hypoxia in the patients.\textsuperscript{[16]} Accumulated data from several studies have shown that recombinant adenoviruses carrying MEL genes with hypoxia response element (HRE)-AFP promoters or survivin promoters (specifically active in tumor cells) are used to selectively express melittin in tumor cells and induce cytotoxicity.\textsuperscript{[17-20]} Further studies are necessary to show if recombinant adenoviruses carrying M-ACE2SBD genes with HRE-AFP promoter scan be used for the treatment of patients infected with SARS-CoV-2.

**CONCLUSION**

In the current study, docking of a designed peptide (M-ACE2SBD) and coronavirus spike glycoprotein-binding domain has received good binding scores. This study has hypothesized that 17 N-terminal amino acids of the mutant melittin used in M-ACE2SBD design are potentially hydrophobic and attach coronavirus-2 through lipid envelope of the virus. Toxicity and hemolytic activity of the melittin hydrophobic motif decrease in comparison to the native melittin due to lack of peptide binding to the exposed anionic lipids of human cell membranes and hence can be recommended as an appropriate drug for clinical uses. However, this fusion peptide may include toxicities on normal cells and safety should be addressed in complementary studies. Furthermore, ACE2SBD has resulted in specificity of the designed M-ACE2SBD for SARS-CoV-2, which attaches to the virus and selectively degrades it.

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**Conflicts of interest**

There are no conflicts of interest.

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