Lopinavir and Ritonavir have a high affinity to SARS-CoV-2 S-protein Receptor-Binding Domain sequenced in Brazil

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected at China in December 2019 and rapid worldwide spread, causing the coronavirus disease 2019 (COVID-19). In this pandemic situation, the importance of structural-functional relationships between virus and host cell should be considered. In this work, we investigated the molecular interactions of seven drugs used in clinical therapy by in silico analysis with specific protein target of SARS-CoV-2 – RBD domain of the Brazilian S protein genome sequence – in docking models. Initially, a three-dimensional structure of SARS-CoV-2 spike glycoprotein receptor-binding domain (RBD) model was obtained by homology. Then, a prediction analysis of cavities in the RBD structure was performed to detect a possible active site in the S protein fragment. Our molecular docking study demonstrated that only 2 ligands showed considerably acceptable values in relation to the seven drugs (Umifenovir, Darunavir, Lopinavir, Ritonavir, Remdesivir, Pirfenidone, Oseltamivir) used to screen. The interaction between Lopinavir and RBD revealed binding affinity of -9.8 kcal/mol and interactions with residues PHE168, GLY167, SER176, GLN175, GLU166, LEU134, LEU137, TYR171, PHE138, LEU174, PHE172. Ritonavir demonstrated binding affinity of -8.9 kcal/mol and interactions with residues ARG148, ASN130, VAL23, SER81, ASN33, PHE29, TYR33, SER31, ASN132, ALA26, ALA30, ALA34, TYR133. Molecular dynamics simulations were performed to evaluate the stability of the complexes formed. The present study shows that protease inhibitors Lopinavir and Ritonavir have best binding at the active site (the RBD of S protein) through molecular docking.

1 Introduction

Coronavirus disease (COVID-19) is a disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a virus identified in December 2019 in Wuhan, China, and is currently recognized by the World Health Organization as a pandemic [1, 2]. To date, SARS-CoV-2 has reached 188 countries worldwide, with more than 519.105.112 infected, including 6,266,424 deaths. In Brazil, the outbreak of COVID-19 outreached 30.682.094 confirmed cases, and 664.872 deaths until May 16, 2022 [3].

The general genome of the first SARS-CoV-2 sequenced in Brazil (Genbank code: MT126808.1) on March 1 (2020) and then made publicly available (one day after) at the National Center for Biotechnology Information (NCBI) the following day [4]. In the viral structure, the spike glycoprotein (S protein) is divided into the S1 and S2 subunits. Among with ACE and TMPRSS2, S protein is one of the SARS-CoV-2 proteins that plays an important role in its entry into the host cell [5]. In the S1 region is the receptor-binding domain (RBD), which represents the domain for interaction with the angiotensin-converting enzyme 2 (ACE2) on the surface of the target cell [6, 7]. This interaction contributes to the stabilization of the S2 domain, important for the fusion of viral and cellular membranes [5, 8]. Due S protein plays a key role in the pathogenesis of SARS-CoV-2, several studies have been directed towards the use of monoclonal antibodies against it and have shown a promising neutralizing effect on viral entry into the cells in vitro, which indicates its ideal vaccine application to prevent COVID-19 [9, 10].
There is still no specific treatment for COVID-19. However, since the beginning of the pandemic, scientists have sought effective vaccines or a treatment using new compounds or, even more, through repositioning of FDA approved drugs [11]. Considering the structural-functional relationships between the S protein of SARS-CoV-2 and ACE2, and its importance to attachment and entry, resulting in replication cycle, the S domain could be a promising target to antiviral drugs. In the present study, we propose an in silico analysis of the molecular interactions of drugs revealed by the FDA with the RBD domain of the Brazilian S omicron protein genome sequence, in docking models, as a strategy for functional evaluating drug repositioning.

2 Methods

2.1 Sequence Alignment and Modeling

Homology modeling is widely applied to create a reliable protein structure using its own amino acid sequence [12]. The complete genome sequence of the Omicron variant of SARS-CoV-2 in Brazil (ON241705.1) is found in the NCBI nucleotide bank (https://www.ncbi.nlm.nih.gov/). The SWISS-MODEL web server (https://swissmodel.expasy.org) was used to build the SARS-CoV-2 S protein RBD model [13].

The structure obtained (PDB ID: 6W41) was used as a model that showed 99.55% similarity to the RBD homologue sequence. The 6W41 structure was obtained experimentally using X-ray diffraction (XRD) with a resolution of 3.08 Å, deposited in March 2020 at the Protein Databank (PDB) website (https://www.rcsb.org/). After obtaining the RBD model, the structure analysis and verification server (SAVES v6.0) from the University of California at Los Angeles (UCLA) was used to test the structure three-dimensionally [14, 15]. The software used to check the model’s validity was PROCHECK [16], Verify 3D [17], PROVE [18] and ERRAT [19]. After the model was validated, structural minimization was performed in the UCSF CHIMERA v1.14 software using the AMBER ff14SB force field [20] and PROSA [21].

2.2 Preparation of Ligands

ChemSpider (http://www.chemspider.com/) is a chemical database that offers fast access to over 67 million of structure-based chemistry information [22]. The ligands Umifenovir (CSID: 116151), Darunavir (CSID: 184733), Lopinavir (CSID: 83706), Ritonavir (CSID: 347980), Remdesivir (CSID: 58827832), Pirfenidone (CSID: 37115), Oseltamivir (CSID: 58540) were obtained from this virtual repository. All structures were optimized using Avogadro 1.2.0 software, pre-calculated with force field using MMFF94 type and geometrically optimized using the Functional Density Theory (DFT) method with B3LYP correlation functional and base 6-31G (d) present in the GAMESS package [22, 23].

2.3 Molecular Docking

In pre-docking, pockets and cavities of the obtained protein were detected through the WEB server CASTp (http://sts.bioe.uic.edu/castp/index.html) with a 1.4 Å radius probe (standard value) for checking possible active sites in the structure [24]. The AutoDock Vina software and AutoDock Tools were used in all docking experiments [25]. In AutoDock Tools, water and other residues present in the structure that
were not critical for the study were removed and then a polar hydrogen group was added to establish connections between the macromolecule and the tested ligand. The Grid box was generated with the coordinates X = -40.0, Y = -39.978 and Z = -6.261 and the size of the box was X = 80, Y = 80 and Z = 94. The binding capacity of the tested ligands and their corresponding binding affinity scores (ΔG) were used to determine better molecular interactions, accepting ΔG ≤ -8.0 kcal/mol for structures that can be suitable candidates for SARS-CoV-2 treatment. The visualization of the binding of the structures was observed by Pymol v2.0 [26] and Edu and LigPlot + v2.2 [27] in 3D and 2D formats.

2.4 Molecular Dynamics

All simulations of molecular dynamics (MD) were performed using GROningen MAChine for Chemical Simulations (GROMACS) versão 5.1.2 [28]. The force field OPLS-AA/L and of TIP3P water model integrated into GROMACS were used to MD simulations [29]. The peptide topology file was prepared by GROMACS, while the ligand topology was obtained from LigParGen. In order to satisfy minimal imaging conventions, the system was initially accommodated in a cubic box with a distance of 2 nm between the protein complex and the box. All binding lengths of proteins and ligands were constrained using the LINCS algorithm while water molecules were constrained by the SETTLE algorithm [30, 31]. A total of 30,000 water molecules were added to a cubic simulation box containing the unbound RBD-Omicron structure, respectively. Each system was energy-minimized using the steepest and most balanced descent algorithm to achieve the proper volume. Interactions without short-range and long-range bonds were calculated by applying double-range cutoff points of 0.9 and 1.4 nm, respectively. The leap frog algorithm with time step 2 fs was used to integrate the motion equation and the neighbor list was updated every five steps. Long-range electrostatics was treated using the Particle Mesh Ewald method, with a Fourier grid spacing of 0.15 [32].

Periodic boundary conditions were applied in all three directions. The balance of the systems was carried out in two main steps. First, the system was gradually heated to 300 K in the NVT set using the v-rescale algorithm for 10 ns. Then the NPT pool was used for 5 ns constraining the complexes (RBD-Omicron/ritonavir, RBD-Omicron/lopinavir) while slowly allowing the solvent molecules to relax around it. Finally, another 10 ns NPT equilibrium was carried out, removing the restrictions on the complexes. For all systems, the mean values of temperature and pressure remained close to the desired values. The balanced systems were then subjected to MD simulations of unrestricted production of 100 ns each, maintaining the target pressure (1 bar) and temperature (300 K). The reproducibility of the results was verified by two different repeated simulations with different initial velocities and equilibrium times. Overall trends in RMSD and RMSF were calculated from MD simulations [33].

3 Results And Discussion

Based on the sequence identity between SARS-CoV-2 and the receptor binding domain (RBD), SWISS-MODEL has built a valid and high-quality model with a 93.85% similarity to a structure of 6W41. Figure 1A shows superposition results. The corresponding regions in the SARS-CoV-2 genome were identified based on sequence identity and similarity score. Figure 1B shows aminoacid sequence between
the constructed structure and the model example structure (223 aminoacids). It is possible to notice the
14 mutations (yellow and green) in the sequence of the RBD of the omicron of Brazil-2022 compared to
the original strain of Wuhan-2019. As a result of the high sequence similarity, the homology models
revealed a surprisingly conserved overall architecture. The overlap of RBD-BR with the RBD (6W41) SARS-
CoV-2 protein demonstrated high structural similarity, with mean square deviation (RMSD) values of
0.35Å. In addition, the average value of the classifier QMEANDisCo [34] Global was of 0.83 ± 0.05
(Fig. 1C). The results confirmed the higher quality of the model, where the default score ranges from 0 to 1 [35].

Then, the modeled structure was validated through PROCHECK, Verify 3D and PROSA II analyses.
PROCHECK analysis predicted that the hypothetical model obtained 89.6% of residues in the favored
region; while 9.1% is residual in the additional allowed region, 1.2% in the generously allowed regions, and
0% in the disallowed regions. The quality factor of the residues from the COVID-19 RBD model when
evaluated by Verify3D showed that 98.26% of the residues had an average 3D-1D score ≥ 0.2, which
represents a good score, suggesting high compatibility of the atomic model (3D) with its amino acid
sequence (1D). The ERRAT Ramachandran plot predicted that the final model had 97.8% residuals in
favored regions, 2.2% in allowed regions, and 0.0% in outliers (Fig. 1D). A comparative analysis was
performed using the ProSA II web algorithm, which predicted that the final structure gained a Z-score of
-5.91 and therefore also falls within the range of scores established for proteins of similar size, with NMR
quality (Figs. 1E and F).

The pathway of virus entry in host cells may be effective targets for treatment. In Fig. 2, we predicted the
conformations of seven therapeutics drugs and its complex with SARS-CoV-2 RBD, blocking the binding
with putative receptor, human ACE2. The tested drugs in this study were Umifenovir, a broad-spectrum antiviral used in the prophylaxis and treatment of influenza and which has an in vitro antiviral effect
against SARS-CoV [36, 37]; Oseltamivir, a neuraminidase inhibitor used to treat influenza [38]; Remdesivir,
a nucleotide analog prodrug used to treat Ebola virus [39, 40]; inhibitors of HIV proteases Darunavir,
Lopinavir and Ritonavir [41]; and the antifibrotic Pirfenidone, an effective drug against idiopathic pulmonary fibrosis [42, 43].

Umifenovir showed, in previous studies, antiviral activity in vitro [35]. Clinical pilot trial was conducted in
Wuhan city, with 36 patients, by analysis of the Reverse Transcriptase-Polymerase Chain Reaction (RT-
PCR), revealed a tendency to decrease of viral load and mortality [44]. Also, a clinical trial was performed
in combination with Lopinavir and Ritonavir, demonstrating promising results [45]. Studies of phase 4 are
ongoing [46]. However, in our results, we observed a score lower (-5.7 kcal/mol), considered a moderate
binding with RBD domain. It means, that its positive results, in initial clinical trials, appear did not involve
the interaction with this domain, as well as could be associated immunological strategies, through
modulation of immune response, that require further investigations.

Oseltamivir, a neuraminidase inhibitor, known as anti-flu drug, was also recently reported as effective
against SARS-CoV-2. Currently, it is under randomized controlled trial, on Phase 3, but there is little
evidence about its mechanism of action [47]. Our analysis showed a lower binding with RBD domain (-4.9 kcal/mol). A previous docking study demonstrated its interaction with papain-like protease (PLpro) and main protease (Mpro). Alone, Oseltamivir had a score lower (-4.65 kcal/mol), while in combination (Lopinavir/Ritonavir) showed a stronger interaction (-8.3 kcal/mol) [48]. An *in silico* study evaluated others targets to SARS-CoV-2 and Oseltamivir did not show any anti-SARS-CoV-2 potential [49].

Other drug evaluated in the present study was the Remdesivir, a newer nucleotide analogue RNA polymerase inhibitor, and showed (-6.0 kcal/mol). When tested against MERS-CoV, presented interaction with non-structure protein 8 (Nsp8) and Nsp12 domains *in vitro* [50]. It was also confirmed that Remdesivir couple’s exonuclease (ExoN) and RdRp (or Nsp12) [51]. RdRp is a crucial viral enzyme in the life cycle of RNA viruses, such as SARS-CoV-2, and Elfiky [52] showed that the Remdesivir has tightly bind to its RdRp (-7.6 kcal/mol). About ACE2 binding site, Remdesivir showed a score lower of energy binding (-5.62 kcal/mol) [53]. However, other targets were evaluated by molecular docking, and showed efficient binding energy for Nsp10, Nsp16, Mpro in apo form COVID-19, and S protein [49].

In animal model of infection, Remdesivir also showed anti-SARS-CoV-1 activity mediated by the viral polymerase and the proofreading exoribonuclease [54]. Additionally, it inhibits efficiently SARS-CoV-2 in rhesus monkey cells culture [44, 55]. Clinical trials are ongoing, and has demonstrated effectiveness for COVID-19 [55] (phase 3 NCT04292730 - March 3, 2020; NCT04292899 March 3, 2020 - and NCT04401579 - March 3, 2020). Based in these findings, the interaction between Remdesivir and RBD domain appear did not able to inhibit the receptor-binding domains of SARS-CoV-2, but its crucial role in RNA synthesis and as well as ExoN (occupant in the N-terminal domain of Nsp14 and decreasing its excision rate) could explain the efficiency in humans.

It was observed that the best affinity (most negative) interactions are below the gray score limitation line (-8.0 kcal/mol). Drugs with that score were able to bind tightly with the RBD of S protein that perform the process of molecular replication of the SARS-CoV-2 and, therefore, are potential candidates for inhibition of the processes and reinforcement of use of those currently in clinical trials showing promising results (a total of 58) [56]. Among tested drugs, these two drugs showed the best results with RBD viral protein docking (-9.8 kcal/mol and - 8.9 kcal/mol, respectively). Darunavir, another HIV protease inhibitor, in turn, did not come out as one of the best docked drugs (-5.9 kcal/mol).

Figure 3 shows the best molecular docking simulation results that followed for residue interaction analysis and molecular dynamics simulation. The hydrophobic interaction residues of the RBD structure are represented by dashed semicircles around the structure. The H bonds are green dashed lines. It is observed that Lopinavir (Fig. 3C) presented 11 interactions, Hydrophobic: ILE8, VAL9, ARG10, PHE11, LEU72, LEU199, LEU200,ALA202, PRO203, ALA204, PHE 223; 1 conventional hydrogen bond to residue CYS73. With the ligand Ritonavir (Fig. 3D), it showed 10 interactions, Hydrophobic: VAL9, PHE11, ASP71, LEU72, CYS73, HIS201, PRO203, ALA204, LYS210, PHE 223; 3 conventional hydrogen bonds with residue ARG10.
The RMSD is a measure of the deviation in the position of the Cα atoms in relation to the initial frame, as a function of the simulation time. An RMSD value of \( \leq 2.00 \, \text{Å} \) suggests that the protein structure remains stable throughout the simulation [57]. The RMSD portions of RBD, RBD-lopinavir Complex and RBD-ritonavir Complex are shown in Fig. 3E. All simulations showed stabilization during the time of 100ns.

An RMSF analysis examines residual fluctuations and flexibility during simulation. The greater the fluctuations, the lower the stability [58]. This parameter is extremely essential to explore the role of the individual amino acid in the stability of any binding protein complex. The RMSF of each amino acid of the RBD-lopinavir (Fig. 3F) and RBD-ritonavir (Fig. 3G) complexes fluctuate almost similarly, with minor variations. Residue CYS073 of the RBD-lopinavir complex was identified in the graph due to its fluctuation of 7.23 Å, this is possible due to its hydrogen interaction shown in Fig. 3C, thus confirming the findings of the residue interaction analysis. While in Fig. 3G we can see the highest fluctuation peak referring to an ARG010 oscillation also confirming the hydrogen bond seen in Fig. 3D.

Since Lopinavir and Ritonavir are protease inhibitors, several studies of repurposing drug have been performed using Lopinavir and Ritonavir alone or in combination. In in vitro evaluation, Lopinavir demonstrated promising results [59], as well as in combination with Ritonavir showed inhibitory effects on SARS-CoV-2 [60]. Computational studies have focused on the evaluation of these drugs in proteases present in SARS-CoV-2. Studies using docking techniques and molecular dynamics have shown a strong connection of these drugs with the SARS-CoV-2 Mpro, presenting free energy of medium binding of -10.89 kcal/mol (Lopinavir) and -14.93 kcal/mol (Ritonavir) [61]. In addition, it was seen that the combination of Lopinavir, Ritonavir and Oseltamivir can improve the free energy values of binding with Mpro, making the system more stable [62]. For Ritonavir was observed efficient binding energy for Nsp10, Nsp16 and S protein, while for Lopinavir was observed to Mpro/3CLpro, ADP ribose phosphatase of Nsp3, Nsp9 and ACE2 receptor protein [49].

For clinical use, the association (Lopinavir/Ritonavir) have been advantageous once Ritonavir, a potent reversible cytochrome P450 3A4 inhibitor, slows the metabolism of Lopinavir, maintaining its plasma concentration [63, 64]. Currently, Lopinavir/Ritonavir are under clinical trials (as phase 3 NCT04303299). However, clinical trials with protease inhibitors have not demonstrated positive interaction reported is enough against infection. Darunavir and Lopinavir/Ritonavir might not improve the clinical outcome in the treatment of mild/moderate COVID-19 [64], and Darunavir in combination with Cobicistat did not provide clinical improvement compared to control [65].

4 Conclusion

The present study shows that the protease inhibitors Lopinavir and Ritonavir can act by binding with the RBD of S protein, a region of extreme importance for the entry of the virus into the cell, modeled after the SARS-CoV-2 genome found in Brazil. Thus, this work can collaborate with future studies to evaluate the mechanism of action of these drugs against SARS-CoV-2. In addition, the importance of these studies in the rational design of drugs must be emphasized since such molecules can be used as the basis for
these studies and knowledge of possible mechanisms of action at the molecular level is extremely important.

**Abbreviations**

ACE2, Angiotensin-Converting Enzyme 2; COVID-19, Coronavirus Disease 19; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; Mpro, Main Protease; NCBI, National Center for Biotechnology Information; Nsps, Non-Structure Proteins; PDB, Protein Databank; RBD, Receptor-Binding Domain; S Protein, Spike Glycoprotein; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

**Declarations**

**Ethical Approval**

Not applied in this study.

**Competing interests**

The authors declare that there are no conflicts of interest, financial or otherwise.

**Authors' contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Helyson Lucas Bezerra Braz, Aline Diogo Marinho, Danilo Galvão Rocha and João Alison de Moraes Silveira. The first draft of the manuscript was written by Aline Diogo Marinho and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this article.

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**References**


Figures
Figure 1

Result of structural modeling by homology of Omicron's RBD structure. (A) The RBD COVID-19 model, built by the Swiss-Model is shown in purple, while the superimposed structure (6W41.1.C) is seen in yellow. (B) Compared sequences of the Brazilian built RBD model (Omicron-RBD) and the structure used in the homologous construction (6W41.1.C). (C) Structural validation chart of the classifier QMEANDisCo referring to RBD-omicron homology. (D) Ramachadran plot of the RBD-omicron structure modeled in 3D.
(E) Result of energy prediction compared to PROSA database structure. (F) Z-score of the RBD-omicron structure.

Figure 2

Histogram with the binding affinity results ($\Delta G$) between drugs and the modeled RBD. The dashed line shows the limit value of -8 kcal/mol.
Figure 3

Results of docking and molecular dynamics evaluations. (A) interaction with Lopinavir in the 3D model. (B) interaction of Ritonavir in the 3D model. (C) Lopinavir in the 2D model. (D) Ritonavir in the 2D model. (E) RMSD plot of the complexes and isolated RBD. (F) RMSF of the RBD-lopinavir Complex. (G) RMSF from the RBD-ritonavir Complex.