Current Trends in Biotechnology and Pharmacy
Vol. 15 (3) 233-240, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.25

Can Structural Differences Between SARS-CoV and SARS-CoV-2 Explain Differences in Drug Efficacy?

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Abstract

The severe acute respiratory syndrome corona virus (SARS-CoV)and severe acute respiratory syndrome corona virus-2 (SARS-CoV-2), both virus spike proteins are recognized by the cell surface receptors, human angiotensin converting enzyme-2 (ACE-2).These viruses gain access into the host cell through ACE-2receptors.The main aim of the current study was to elaborate on the structural differences in the receptor binding domain (RBD) of spike glycoprotein in SARS-CoV and SARS-CoV-2 that bind at the same active binding site. The crystal structures of receptor bound spikes of SARS-CoV and SARS-CoV-2 were compared using UCSF Chimera and pyMOL software which revealed significant differences in the receptor binding domain of the spikes with variation in the amino acid residues. It was also observed that conformational changes occurred in the amino acid residues at the binding site on ACE-2 receptor. These conformational changes in ACE-2 binding site of SARS-CoV-2 were attributed to a greater number of contacts forming between RBD and active binding site when compared to that of SARS-CoV and could explain any differences in the effectiveness of drugs against SARS-CoV and SARS-CoV-2. In addition, using Autodock vina software, drugs that were found to be effective in SARS-COV treatment were docked at active binding site on ACE-2.Antivirals, ACE-2 inhibitors and corticosteroids were docked at the active binding site domains of ACE-2 receptor in SARS-CoV andSARS-CoV-2. Antivirals such as Oseltamivir, Umifenovir, Favipiravir, Remdesivir and antibiotics such as Moxifloxacin and Azithromycin, Ace-2. Antivirals inhibitors such as Losartan and steroids such as Dexamethasone have shown a greater negative docking score (indicating more binding affinity) in and SARS-CoV-2 when compared to that of SARS-CoV. This kind of preliminary analysis using computational techniques could help in screening and repurposing the existing drugs that are potential in treating new diseases such as CoVID-19.

Keywords SARS-CoV-2, ACE-2 receptors, RBD, Docking, Score.

Introduction

The viral infections continue to emerge and pose a serious public health issue. The present pandemic is caused by corona virus belonging to the genus β corona virus of corona viridae family. The disease started as an outbreak with pneumonia like respiratory disease symptoms. So far there were six corona viruses that infected humans and the novel corona virus (Covid-19)was the seventh human corona virus. Around the world people commonly get infected by human corona viruses such as 229E, NL63, HKU1, OC43. These four human corona viruses cause mild to moderate flu like symptoms (1). Till date worldwide deaths due to COVID-19 have reached up to 2.1 millionand continue to increase daily (2).

Corona viruses that infect animals, sometimes evolve as human corona viruses, which include severe acute respiratory syndrome corona virus (SARS-CoV), Middle east respiratory syndrome (MERS-CoV)and the present severe acute respiratory syndrome

corona virus-2 (SARS-CoV-2/Covid-19) making it the third zoonotic corona virus.Unlike the other corona viruses these three viral infections cause severe symptoms ranging from severe respiratory distress to death.

The SARS-CoV-2 has many similarities with that of SARS-CoV, as both cause respiratory illness, spread of disease is by contact and via the droplets produced by sneezing or coughing by an infected person, recognized by angiotensin converting enzyme-2 (ACE-2) receptors (3). Both these virus's genome consists of an enveloped single stranded positive RNA (4).Various studies reported that virus gains access to the host cell is via binding to cell surface receptors. The SARS-CoV -2 was known to bind to ACE-2 receptors on the surface of the respiratory epithelial cells.The structural analysis of receptor bound SARS-CoV and SARS-CoV-2 spike proteins revealed the active binding spots on ACE-2 receptors (5).But the drugs that were widely used and effective in treating SARS-CoV such as, Ribavarin, Methyl prednisolone, levofloxacin were ineffective in case of SARS-CoV-2.

The present study focuses on the analysis of differences between the spike proteins of SARS-CoV and SARS-CoV-2, their binding sites on human ACE-2 receptors and the conformational changes at the binding sites. In addition, various drugs that were effective in SARS-CoV such as anti virals, ACE-2 inhibitors and corticosteroids were docked at the spike receptor binding sites on ACE-2 receptor and compared the drug binding affinities to address why the drugs used in treating SARS-CoV failed to treat COVID-19.

Materials and Methods

To study the structural features and analyze the binding affinity of drug moieties, three different software were used– namely, UCSF Chimera, pyMOL and Autodock vina. The crystal structures of ACE-2 receptor bound to the virus spike glycoprotein were obtained from the protein data bank. SARS-CoV: PDB code=3SCI and SARS-CoV-2: PDB code=6VW1.

The 3D structures of drug moieties were obtained from pubchem data base.The crystal structures of SARS-CoV and SARS-CoV-2were overlapped using UCSF Chimera and the RMSD values were calculated.The interface interactionsbetween the spike RBD and ACE-2 receptor were analyzed using pyMOL software. RMSD (Root mean square deviation) is often used to measure the quality of reproduction of a known binding pose.A low RMSD with respect to a true binding pose is good.Ideally less than 1Aº.

RMSD=√((Σ_i d_i^2)/n)

Where, d=distance between each of the n pairs of equivalent atoms in two optimally superposed structures.The RMSD value is '0' for identical structures.The values increase as the two structures become more different. Docking was performed by Autodock vina and the resulting files were analyzed tostudy of type and strength of interactions between drug molecule and ACE-2 receptor using pyMOL. The result of docking was obtained as docking score, which is a mathematical function used to approximately predict the binding affinity between two molecules after they have been docked. This score mimics the potential energy change when the protein and ligand come together. The greater the negative score the stronger is the binding affinity.

Results and Discussion

The first objective of the study was to examine the crystal structures of SARS-CoV and SARS-CoV-2spikes bound to ACE-2 receptors and visualize the structural differences.Majority of the crystal structure between SAR-CoV and SARS-CoV-2 spike bound to the ACE-2 receptor was be similar, except for the loop of the spike at the interface (Fig-1A,1B). The RBDs of spike proteins of SARS-CoV and SARS-CoV-2 consists of 174 and 194 residues, respectively.Overlapping the spike RBDs using UCSF Chimera and an evaluation across all 171

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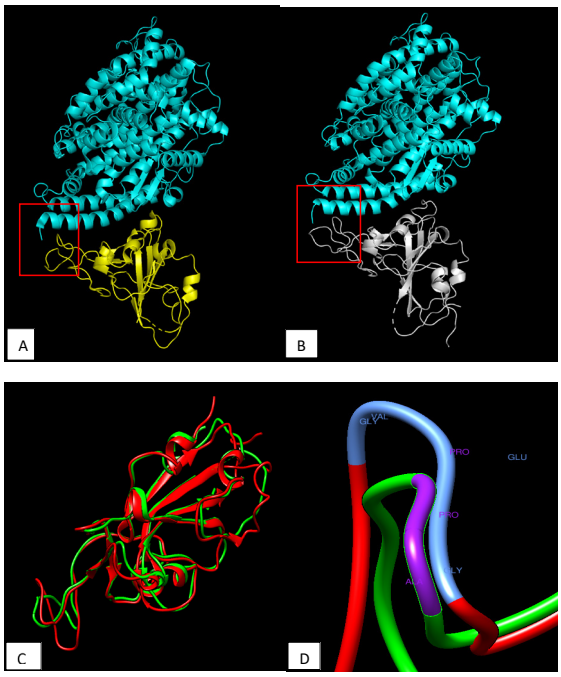

Fig1. (A). SARS-CoV RBD binding to ACE-2, (B). SARS-CoV-2 RBD binding to ACE-2and highlighted area in the box shows the variation of distance between loop and N-terminal helix of ACE-2. (C). overlap of RBD-SARS-CoV(green) and SARS-CoV-2 (red), (D). Amino acids at the loop in purple for SARS-CoV and in blue for SARS-CoV-2.

superposed residues showed an overall RMSD of 2.011 and 63.59 percent identity. There was a variation at the loop that interacts with ACE-2 receptor. The loop in SARS-CoV consists of proline-proline-alanine, a three amino acid motif, where the tandem prolines (Fig-1D) take a sharp turn. In case of SARS-CoV-2 the loop consists of four amino acids, glycine-valine-glutamine-glycine. This extra amino acid in the loop and the two flexible glycine led to a wider loop allowing it to form more interactions with the ACE-2 receptor. In case of all corona virus RBDs the distance between the two-disulfide containing cysteine residues was crucial. Due to these structural differences, extra hydrogen bonds were formed between the main chain of ACE-2 and asparagine-487 and alanine-475 of the SARS-CoV-2 RBM.As a result of these hydrogen bonds with the main chain, the ridge takes a more compact conformation and the loop with alanine -475 gets closer to the AEC-2. The consequence of this moving of the loop closer led to a greaternumber of contacts with the N-terminal helix of ACE-2. As shown in figure-3, clearly indicated the interactions that led to a more compact fit on to the binding site(6).

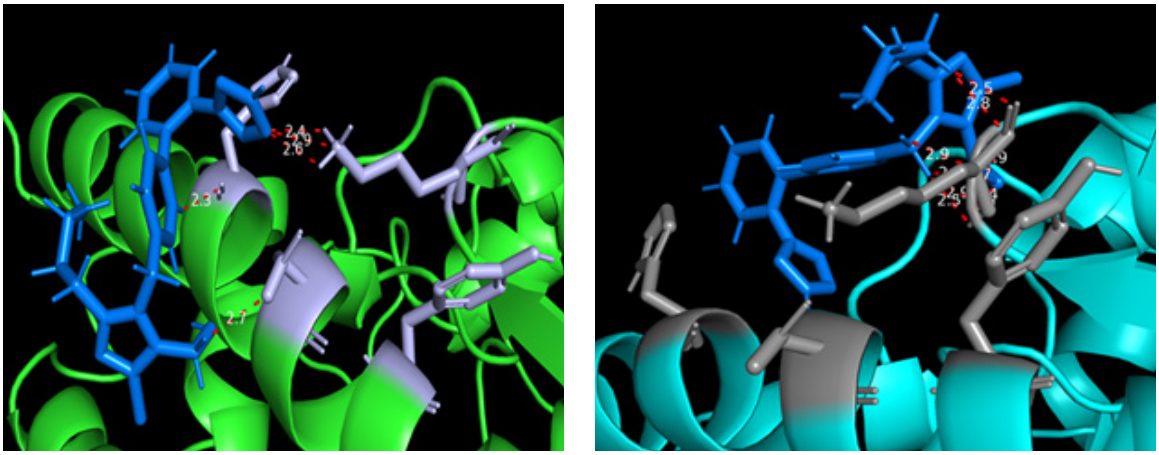

Fig. 2. Docking of losartan (in blue) at the RBD binding site residues (in grey) of ACE-2 crystalstructure in (A) SARS-CoV and (B) SARS-CoV-2.

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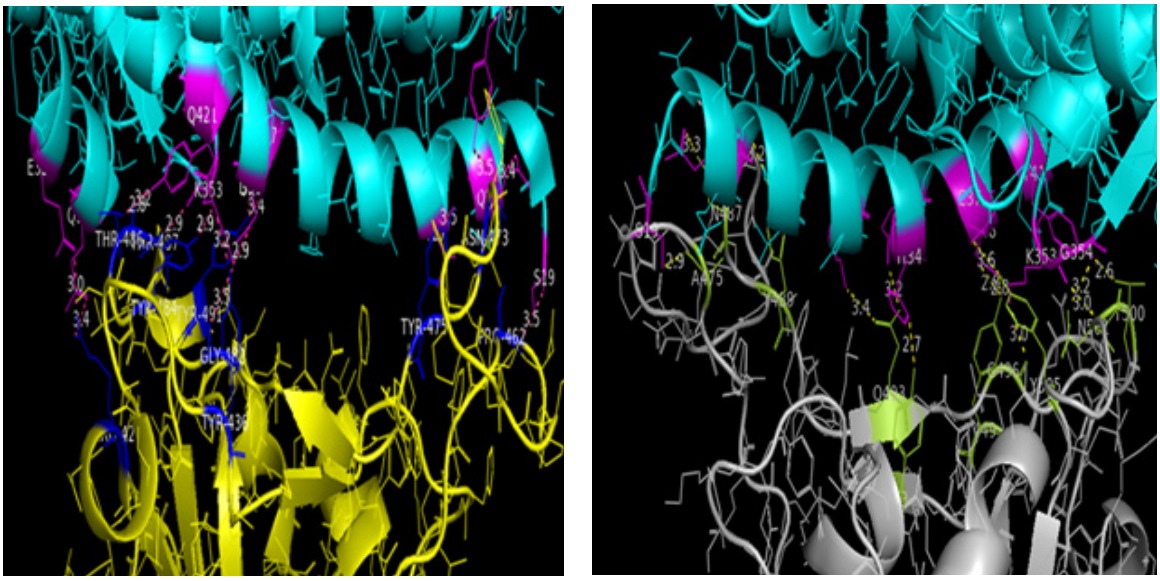

Fig. 3.The ridge in the SARS-CoV-2 RBD (right)forms more contacts with the N-terminal helix of ACE-2.

The ACE-2 receptor binding domains in crystal structures of SARS-CoV and SARS-CoV-2 were overlapped. An evaluation across all 588 superposed residues showed an overall RMSD of 1.382 and a 96.82 percent identity of the sequences, indicating certain conformational changes at the binding sites. The ACE-2 receptors have two hotspots for RBD binding. One hot spot includes tyrosine-41 and the other lysine-353.All the interactions at the interface of ACE-2 and the RBD of SARS-CoV and SARS-CoV-2 within a range of 3Aº as shown in Table 1. Initially a cutoff distance of 4Aº was selected which included all polar and π-interactions. To exclude the weaker interactions amongst these, a cutoff distance of 3Aº was selected.

Table1. Interface interactions between RBD of spike protein and ACE-2 receptors.

SARS-CoV			SARS-CoV-2		
ACE-2 Residues	RBD Residues	Bond length (Aº)	ACE-2 Residues	RBD Residues	Bond length (Aº)
Glutamine-24	Asparagine 473	2.7	Serine-19	Alanine-475	2.6
Aspartic acid -38	Tyrosine -438	2.6	Lysine-31	Glutamine-493	2.9
			Glutamic acid-35		2.9
Tyrosine-41	Threonine-486	2.4	Aspartic acid-38	Tyrosine-449	3.0
Glutamine-42	Tyrosine-484	2.9	Tyrosine-41	Threonine-500	2.6
Lysine-353	Threonine-487	2.9	Tyrosine-83	Asparagine -487	2.9
Aspartic acid-355	Thronine-486	3.0	Lysine-353	Glycine-496	3.0
				Glycine -502	2.8

Table2. Bond length and interactions between losartan and lysine 353 of ACE-2 receptors.

	SARS-CoV	SARS-CoV-2
Number of interactions between losartan and lysine-353 with in 3Aº	3	9
Bond lengths	2.4, 2.6, 2.9	2.2, 2.4, 2.5, 2.8, 2.8, 2.9,2.9, 2.9, 2.9

Table3. List of drugs binding at ACE-2 receptors with their docking score.

Drugs	Score		Effectiveness in Covid-19
		SARS-CoV-2	
Antibiotics			
Levofloxacin	-5.2	-5.0	Not effective
Moxifloxacin	-4.6	-5.1	Effective (7).
Hydroxy chloroquine	-4.3	-4.5	Nosignificant outcomes(8, 9, 10).
Corticosteroids			
Methyl prednisolone	-6.1	-3.9	Not effective
Dexamethasone	-6.6	-6.4	Treats pneumonia & hyperinflammatory syndrome. (11) Decreased mortality rate by 1/3 in patients requiring ventilator (12).
prednisone	-5.7	-5.8	Treats pneumonia & hyperinflammatory syndrome(13).
ACE-2 inhibitors			
Telmisartan	-6.1	-5.8	----------
Enalapril	-4.9	-6.9	No significant outcome(14).
Losartan	-4.4	-5.9	Decrease in mortality rate(15).
Valsartan	-5.0	-5.5	----------
Antivirals			
Remdesivir	-5.2	-5.7	Shortens the time of recovery(16).
oseltamivir	-3.9	-4.5	Early administration decreases the intensity of symptoms(17).
Umifenovir	-4.2	-6.2	Reduces viral load and inhibits spike protein trimerization(18).
Ribavirin	-5.5	-5.0	Not effective
Favipiravir	-4.3	-4.8	Decreases viral load(19).
Macrolides			
Azithromycin	-3.5	-8.5	Shows antiviral and immunomodulatory effects(20).
Calcium channel blocker			
Ipratropium	-4.9	-6.4	----------

Structural differences between SARS-CoV and SARS-CoV-2

The RBD in SARS-CoV-2, forms two additional bonds at N-terminal helix with serine -19 and tyrosine-83 leading to change in conformation of residues at the binding site. The conformational changes in the residues at the binding site were explained by docking drugs at the selected residues on the binding sitesof ACE-2 receptors in both SARS-CoV and SARS-CoV-2.The difference in binding when losartan an ACE-2 inhibitor is docked is clearly visible as shown in Figure 2. In case of the ACE-2 receptor in SARS-CoV crystal structure (Fig-2A) losartan was found binding to histidine 34, aspartic acid 38 and lysine 353. The drug binds to the terminal hydrogens of lysine 353. Whereas in case of ACE-2 receptor in SARS-CoV-2 crystal structure (Fig-2B)was found to bind with only lysine 353 but not to the highlighted terminal hydrogens.

This difference in thebinding could be attributed to the conformational changes occurred at the binding sites on ACE-2 receptor.

The bond lengths of the interactions between losartan and lysine 353 in Table-2 shows that losartan has a greater number of interactions with lesser bond lengths in SARS-CoV-2 when compared to SARS-CoV indicating strong interactions. The docking score of losartan at ACE-2 receptor of SARS-CoV-2 is -5.9 and that of SARS CoV is -4.4, indicating its greater binding affinitytowards ACE-2 receptors in SARS-CoV-2.

Various drug moieties belonging to antivirals, ACE-2 inhibitors, corticosteroids, macrolides, and antibiotics were docked. The obtained docking score was utilized to study why certain drugs were ineffective and which drugs might show some potency in the present COVID-19 treatment scenario.The drugs that were docked at the RBD binding site in ACE-2 receptors with the docking score and the clinical outcomes from various studies conducted are shown in Table 3.Most of the drug moieties have shown a greater negative score when docked at ACE-2 receptors in SARS-CoV-2 when compared to that of SARS-CoV, indicating higher binding affinity. All the drugs that have shown a high binding affinity in case of SARS-CoV-2were found to bind at lysine353 with greater number of interactions. Levofloxacin which forms only one interaction with lysine 353 was ineffective, whereas moxifloxacin forms seven bonds was effective.

Drugs having a greater negative score than -5.0 were found to be effective in SARS-CoV-2 by either reducing the viral load or by treating the associated pneumonia or both. There are a couple drugs which have a score less than -5.0 but were found to decrease viral load and intensity of symptoms associated with SARS-CoV-2.Favipiravir and oseltamivir have scores of -4.8 and -4.5 respectively when docked at ACE-2 binding residues inSARS-CoV-2, which are comparatively greater than -4.3 and -3.9 in SARS-CoV respectively. Drugs such as valsartan and ipratropium have shown a greater negative score and stronger binding at lysine 353 residue, yet no clinical data available to corroborate their potential in COVID-19 treatment.

Conclusion

The studies conducted on crystal structures of ACE-2 bound spike RBDs of SARS-CoV and SARS-CoV-2 using UCSF Chimera, pyMOL and Autodock vina revealed the structural differences in the binding spots and conformational changes at the receptor binding sites between the two viruses. The drugs that yielded a more negative docking score, greater than -5.0were observed to have potential in reducing viral load and associated pneumonia. As pandemic disease like COVID-19 strike the humanity, a preliminary analysis with computational methods could help in the design of new treatments or repurposing of the current treatments.

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