Comparative Docking Studies on Curcumin with COVID-19 Proteins

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Abstract: Corona virus disease 2019 (COVID-19) is caused by a Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), which is a positive strand RNA virus. The SARS-CoV-2 genome and its association to SAR-CoV-1 vary from ca. 66% to 96% depending on the type of betacoronavirdeae family members. With several drugs, *viz.* chloroquine, hydroxychloroquine, ivermectin, artemisinin, remdesivir, azithromycin considered for clinical trials, there has been an inherent need to find distinctive antiviral mechanisms of these drugs. Curcumin, a natural bioactive molecule has been shown to have a therapeutic potential for various diseases, but its effect on COVID-19 has not been explored. In this study, we show the binding potential of curcumin targeted to a variety of SARS-CoV-2 proteins, *viz.* spike glycoproteins (PDB ID: 6VYB), nucleocapsid phosphoprotein (PDB ID: 6VYO), membrane glycoprotein (PDB ID: 6M17) along with nsp10 (PDB ID: 6W4H) and RNA dependent RNA polymerase (PDB ID: 6M71) structures. Our results indicate that curcumin has high binding affinity towards nucleocapsid and nsp 10 proteins with potential antiviral activity.

Keywords: Curcumin, COVID-19, nucleocapsid phosphoprotein, membrane glycoprotein, antiviral mechanism

Introduction

COVID-19 has caused unprecedented morbidity and mortality across the globe (who.int). SARS-CoV-2 contains 29 proteins that includes 4 structural proteins - Spike, Envelope, Membrane and Nucleocapsid, and the rest non-structural and adjunct proteins. A polyprotein is acted upon by an encoded protease giving rise to 16 of these proteins. Whereas the viral RNA genome is close to 30 Kb in size, the structural proteins are mainly involved in entry, replication, assembly and propagation of the virus into lung and other cells carrying the ACE2 receptor (Chen et al., 2020)

A large number of potential therapeutic molecules that include antibiotics, antivirals, and anti-malarials are being tested against COVID-19, that has caused global devastation. In a recent study, Sampangi-Ramiah et al., 2020, have evaluated 27 natural compounds for binding affinities to both the proteases of COVID-19. A few compounds have been found to have good binding affinities ranging from -6.4 kcal/mol (for coriandrin) to -8.0 kcal/ mol (for glabridin) and -8.1 kcal/mol (for glucobrassicin), comparable to the anti-HIV drug saquinavir. In the present study, we have evaluated the binding affinities of 14 drug candidates with SARS-Cov-2 proteins: spike glycoproteins (PDB ID: 6VYB), nucleocapsid phosphoprotein (PDB ID: 6VYO), membrane glycoprotein (PDB ID: 6M17) nsp10 (PDB ID: 6W4H) and RNA dependent RNA polymerase (PDB ID: 6M71) structures. We find that the natural molecule, curcumin from turmeric, has good binding affinities to nucleocapsid and nsp-10, comparable to those of ivermectin, azithromycin and remedesivir. Turmeric as a spice is used extensively in India and is described in ancient literature for its wide medicinal use even as numerous studies describing that food supplements and nutraceuticals may turn out to be long-term option to prevent the viral infection. For example, curcumin has been shown to have a strong anti-oxidative and anti-inflammatory properties and has potential therapeutic effects on chronic diseases (Padmanaban et al., 2018), Noroviruses (Yang et al., 2016), Hepatitis B infection (Herari et al., 2018) and diabetes mediated cererbal infarction (Xia et al., 2018). In addition to its profound immunomodulatory effects, it may also bring about changes by directly binding to crucial viral proteins and preventing their function. Hence, we attempt to understand the molecular interactions and decipher the role of curcumin with SARS-CoV-2 structural and non-structural proteins. To provide molecular details of ligand recognition and better understanding of the ligand binding behaviour of different targets/receptors, we determined characteristic features on ligand binding, safe dosage, K_i values, and classify key factors that direct the docking complexes of drugs. The spike glycoproteins of SARS family to which SARS-CoV-2 and SARS-CoV belong to, have structure resemblance with 8 amino acids related to 14 binding residues, and are known to be conserved in SARS-CoV-2 (Walls et al., 2020). It was further shown that angiotensin converting enzyme-2 (ACE-2) interacts with the binding residues of SARS-CoV-2 (Cynthia et al., 2020, Abdul et al., 2020). We deliberate on the role of curcumin, its antiviral drug

binding efficiency and calculate the dosage concentration using molecular modelling approaches targeted for therapeutics of SARS-CoV-2.

Material and methods

Preparation of ligands: The molecular docking studies were performed using AutoDock 4.2 software (Morris et al. 2009). For the current study, 15 ligands were considered based on their current experimentation in treatment of COVID-19 patients, based on known potent antiviral/antimalarial properties and their plasma concentration (supplementary table 1). The ligands with 3D structures were retrieved from PubChem as sdf files, as others were subjected to a 3D structure generation on CORINA (MN-AM.com) using their SMILE nomenclature. Further, pdbqt files for the ligands were generated by OpenBabel (O'Boyle et al., 2011).

Preparation of proteins and grid parameters: The protein data bank (PDB) structures of different COVID19 proteins were retrieved from RCSB membrane protein (6M17), polymerase (6M71), spike (6VYB), nucleocapsid (6VY0) and nsp10 (6W4H). The protein structures were visualized on PyMOL 1.3 or 2.5 (DeLano, 2002) by demarcating the transmembrane regions if any, present in the protein. While the pdbqt files were generated for the proteins to initiate the grid parameters, we considered less than 1Å and x, y, z coordinates to establish the size of the protein for docking studies. MGL tool 1.5.6 (Morris et al., 2009) was used for generating necessary protein and ligand files along with the respective grids and dock files. The grid generation and docking were subsequently executed through AutoGrid 4.2 and AutoDock 4.2 respectively (Morris et al., 2009). To find the binding affinity of ligands with selected proteins, drug targets were screened from estimated free energy of binding and inhibition constant (K_i) at 298.15 K temperature. The docked ligand-protein complexes were visualized on Chimera 1.12 (Pettersen et al., 2004) for image construction. The binding energies and affinities for the ligands were obtained from the log files of the docks generated by AutoDock. A brief methodological overview is presented in Figure 1.
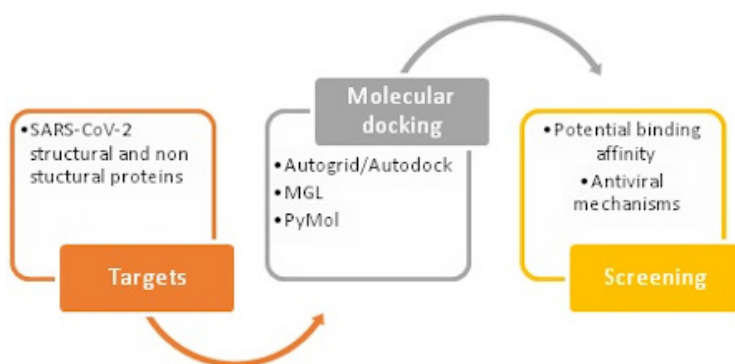
Figure 1: A pictorial methodology outlining molecular docking approaches

Results and Discussion

Comprehensive docking studies were carried out on 14 drug molecules with antiviral properties that are considered for clinical trials along with curcumin using AutoDock 4.2. This resulted in free energy (ΔG) and inhibition constant (K_i) values showing the binding interaction of the ligand molecules with different structural and non-structural proteins of SARS-CoV-2. From the docking studies, we observe that curcumin shows good binding affinity with nucleocapsid (ΔG = -8.75 kcal/mol, K_i = 0.39µM), nsp10 (ΔG = -7.85 kcal/mol, K_i = 1.77 µM) which is comparable to quinidine with nucleocapsid (ΔG = 11.74 kcal/mol, K_i = 2.50 nM) and nsp10 (ΔG = -8.45 kcal/mol, K_i = 639 nm), ivermectin with nucleocapsid (ΔG = -7.11 kcal/mol,K_i = 6.17 µM) and nsp10 (ΔG = -9.82 kcal/mol, K_i = 63.20nm), azithromycin with nucleocapsid (ΔG = -8.71 kcal/mol, K_i = 0.41 µM) and nsp10 (ΔG = -8.03 kcal/mol, K_i = 1.29 µM) in nsp10), and remdesivir with nucleocapsid (ΔG = -6.30 kcal/mol, K_i = 23.94) and nsp10(ΔG = -6.54 kcal/mol, K_i = 16.02 µM) (Figure 2; Supplementary table-2). Ivermectin showed the best affinity towards all the targeted proteins studied and significant efficient binding to the non-structural proteins. The efficient binding to non-structural proteins is not much in the case of chloroquine, efavirenz, favipiravir and tenofovir, disoproxil.

Curcumin is a molecule with symmetrical halves containing a ring at both the edges (Supplementary figure 1). The bendable chain and the rigid rings of curcumin would serve a good clamp or a hook formation on the loop regions of targeting SARS-CoV-2 proteins. On the other hand, loops are known to play a major role in the stability of the protein structure (Balasco et al., 2013) and hence a molecule binding to loop is a potential modulator of stability. Curcumin does not show a remarkably high C-$_{max}$ value (Cas and Ghidoni, 2019) but the K_i values correlate with binding to nucleocapsid and nsp10 proteins. We have visualised it using PYMOL and identified the best pose of curcumin with binding residues at L161, Q163, A173 of Loop 2 in nucleocapsid protein. We argue that the binding residues of loop 2, _viz._ Q163, L161, A173 in nucleocapsid protein may involve in a distinct signalling system associated with vertebrate evolution, as evident from the complex interaction pathways (Figure 2, panel). We have also identified K4281, L4365 of zinc finger groove in nsp10 protein as the binding residues. The zinc binding sites in nsp10 protein are HIT-type zinc finger family that are reported to bind to transcription regulatory proteins which help in recognizing RNA and also in protein-protein recognition (Su et al., 2006). Consequently, nsp10 plays a major role in viral transcription wherein nsp14 3'-5'

exoribonuclease and nsp16 2'-O-methyltransferase are stimulated by playing a lead role in viral mRNAs cap methylation (Bouvet et al., 2012).
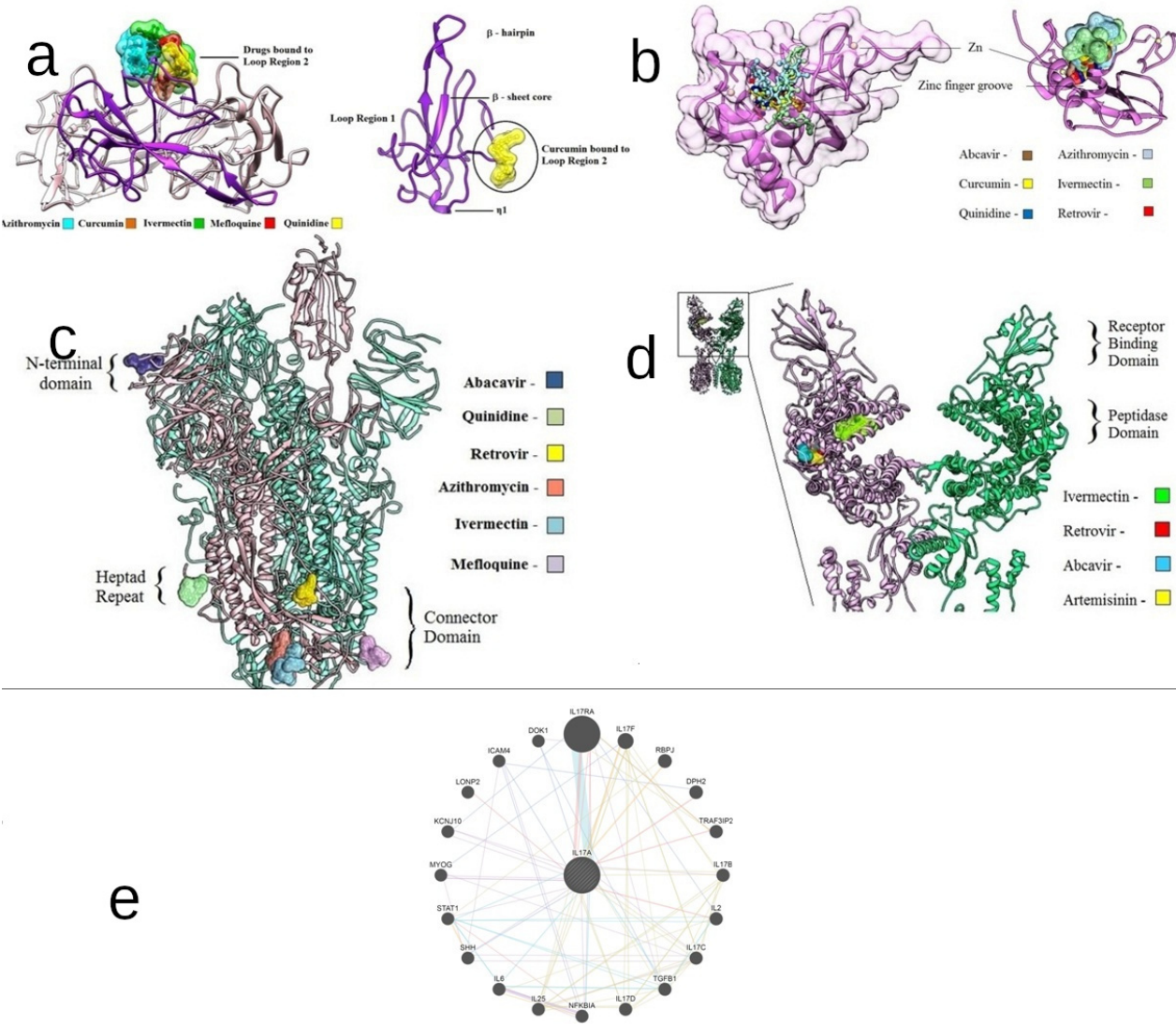

Figure 2: Protein-ligand interactions of (a) nucleocapsid phosphoprotein (PDB ID: 6VYO) (b) nsp10 (c) spike glycoproteins (PDB ID: 6VYB) and (d) membrane glycoprotein (PDB ID: 6M17). (e) Protein interaction map of interleukin-17, a human homologue of nucleocapsid which acts as a potent proinflammatory cytokine produced by activated memory T cells

While nucleocapsid protein regulates replication and transcription of viral RNA, it also inhibits the EF1α-mediated protein translation, altering the cell cycle and further leads to apoptosis in host cells (McBride et al.,

2014, Hilgenfeld 2014, Zhou et al., 2008). From our studies, we demonstrate that the drugs such as azithromycin, mefloquine, quinidine, ivermectin and curcumin show hydrogen binding interactions with Q163, A173, Q70, Q163, G164, L161, T165, L167 in loop 2 which serves as latent targets, activating T-cell cytotoxicity for candidate drug therapeutics. The crystal structure of the RNA binding domain of nucleocapsid protein of SARS-CoV-2 was recently published (Kang et. al., 2020) which details the groove between the palm (loop region 2) and finger region (bsheet core dominantly) at the RNA binding. As loops are known to play a major role in the stability of a protein (Balasco et. al., 2013) they are flexible, and can relatively move with ease. We could, hence, predict the role of this loop in affecting the binding of RNA to the palm groove of nucleocapsid (Figure 2). Curcumin has shown exceptional binding affinity to this region, which therefore makes it a potent target drug for RNA binding domain or nucleocapsid of SARS-CoV-2. On the other hand, nsp16, which methylates the RNA cap with its S-adenosyl-L-methionine dependent methyl transferase activity, requires nsp10 as its stimulatory factor (Chen et al., 2011) as demonstrated in SARS-CoV, and the same is expected to be established in SARSCoV-2. At the same time, inhibiting nsp10 would not allow the viral RNA to camouflage with the eukaryotic RNA of humans and eventually inhibit the replication of virus in the host cell. Among six other drug molecules, we observed that curcumin also shows a very strong affinity to nsp10 and less than 2 mM of the same is adequate concentration correlated with its K_i constant. The binding of all these drugs is observed on the loop in the groove between the zinc fingers. Such strong affinity indicates that it is a probable target site for COVID-19. Thus, curcumin acts as a potential inhibitor of the nucleocapsid (6VYO), which is a multifunctional pro-inflammatory protein (McBride et al., 2014). The regions are also exposed further considering them as epitope targets and is in agreement with the reports that curcumin, nanocurcumin inhibits transcription factor NFκB thereby leading to instability of pro-inflammatory cytokines such as interleukins and TNF-a (Claser et al., 2011). Furthermore, curcumin is reported to suppress components of cellular signalling transduction pathways that play a key role in infected cells growth, and transformation by inhibiting protein kinases, activating enzyme cyclooxygenase (COX)-2 (Prasad et al., 2014). Studies related to spike proteins through their S^B domain (at the N-terminus) interact with ACE2 to make an entry in the host cell (Walls et al., 2020). This makes spike proteins, and more precisely the S^B domain, a hot spot for therapy. Other two important regions of spike proteins are heptad repeats (HR1 and HR2), involved in viral fusion (Bosch et al., 2003), and connector domain, which connects the two heptad repeats (Wrapp et al., 2020) and it helps in stabilizing the post fusion structure (Walls et al., 2017). We obtained six ligands keeping less than 50 μM K_i value as the criteria. Curcumin although shares site with mefloquine, is not a good inhibitor of the Spike protein, but binds to the loop of the connector domain of spike protein with a fair binding energy of -4.9 kcal/mol corresponding to ~250 mM K_i value. Curcumin falls in the top 3 hydrophobic molecules with a logS value making it one of the best drug candidate molecules (Supplementary table 4). The spike proteins and membrane proteins readily come in contact with the cytoplasm of the host cell and therefore would have to come across a higher concentration of the drugs administered to the patient as compared to the proteins inside

the coat (nucleocapsid, nsp10 and RDRP) given that the drug has to cross the surface protein and lipid layer of the virus. Taking into account of this fact, we observed that most of the drugs do not have a C_{max} value of more than 5 mg/mL, even the smallest compound with a molecular weight of ~100 g/mol, e.g. favipiravir (~150 g/mol), would be found at a concentration of 50 mM (the actual C_{mzx} of favipiravir is ~40 mg/mL). Of that, if 5% of the drug manages to reach inside the viral coat, it would account to 2.5 µM of the drugs available to act on nucleocapsid, nsp10 and/or RDRP, hence making the cut-off value for core proteins.

Ivermectin and azithromycin both have a complex structure with multiple rings and they bind at the same locus with similar affinities at the C-terminal of the spike protein. These drugs show interaction with the connector domain, indicating their stability and transition from pre-fusion to post fusion state rather than a preferable ACE2 receptor blocker. The *in silico* docking studies revealed considerable affinity towards a few drugs, *viz.* abcavir, artemisinin, ivermectin and retrovir at the peptidase domain of ACE2 segment of the complex. Whereas the viral domain (B^0AT1) showed considerable affinity only towards ivermectin ($\Delta G = -7.66$, $K_i = 2.44$ mM), yet that was not at the contact site of ACE2 and B^0AT1. We further argue that ivermectin is relatively a complex molecule with reasonably high number of rings, with least C_{max} and relatively most insoluble compound among the ones used here. The low K_i values observed for ivermectin correlates with its low physiological C_{max}, which therefore could make a viable target drug. Curcumin, although has a binding site on both the human domain and the viral domain of the complex, it would need 10 times more concentration to act on them, given its C_{max} value. A binding at the linker region would have been of great value for the therapeutics purpose, which was not observed for the given molecules. In addition, binding of these drugs even at the peptidase domain of ACE2 would inhibit the rotation of the same and hence the change between open and closed conformation, which facilitates the viral attachment (Yan et al., 2020). Receptor binding domain (RBD), above the peptidase domain of ACE2, is identified only in the closed state, probably indicating the role of closed state in anchoring, hence the necessity of transition between the conformations for the invasion.

Conclusions

We propose curcumin as a therapeutic target for anti-corona virus drug development. In this work, we screened 14 ligands against SARS-CoV-2 structural and non-structural proteins and evaluate the binding affinity of curcumin to that of other known drugs that are commercially available. From the current study, it is evident that curcumin could play a major role in regulating the activity of nucleocapsid and nsp10, both of which are related to the detection and processing of viral RNA. At very high concentrations, curcumin acts on the spike and membrane proteins and hence effect on invasion itself is difficult to achieve with the given solubility and plasma concentrations of curcumin. Further study on a combinatorial administration of drugs with curcumin could demonstrate synergistic effects. Despite extensive studies and clinical trials for a variety of diseases, mostly cancers, there has been a view that curcumin is not an ideal drug candidate in view of its

low bioavailability and stability in plasma (Nelson et al 2017). However, curcumin has been shown to be an effective adjunct drug or a food supplement with many strategies reported to enhance its bioavailbility (Prasad et al., 2014). Nevertheless, even with low bioavailbility it is a very effective immunomodulator to cure experimental malaria (Vathsala et al., 2012), cerebral malaria (Dende et al., 2017) and infectious diseases (Padmanaban et al., 2017). The present study shows that in addition to being a potent immunomodulator, it can effectively bind and inhibit the nucleocapsid and nsp 10 proteins of COVID-19. Curcumin can be an ideal adjunct drug, being a food additive and non-toxic to humans at a dosage as high as 8 g/day (Soleimani et al., 2018).

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