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The disruption of SARS-CoV-2 RBD/ACE-2 complex by Ubrogepant Is mediated by interface hydration

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Abstract

Background

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global pandemic affecting approximately 490,000 people and accounting for more than 22,000 deaths and has no generally acceptable cure. Here, the recently resolved 3D structure of SARS-CoV-2 receptor binding domain (RBD) in complex with its receptor-the angiotensin converting enzyme-2 (ACE-2) have provided the basis for screening chemical database for novel entry inhibitors.

Methods

Molecular docking protocols have been used to rapidly screen FDA database for high affinity interaction at the SARS-CoV-2-RBD/ACE-2 interface. One of the top candidates, ubrogepant has been selected and further studied using atomistic molecular dynamics simulation method.

Results

Molecular docking result showed that ubrogepant (UBR) and darunavir have binding energies of -10.4 kcal/mol. MMPBSA free energy analyses of UBR bound to RBD, ACE-2 and RBD/ACE-2 revealed RBD/ACE-2 > ACE-2 > RBD preference. Network analysis showed that interaction captured in the crystal structure were disrupted in UBR-bound state, hydration of the interface and increased atomic fluctuation within the RBD oligomerization interface and ACE-2 zinc binding site.

Conclusions

The ability of ubrogepant to rupture the interaction at the RBD/ACE-2 interface residues of SARS-CoV-2 RBD/ACE-2 complex may result in loss of protein function with direct implication on oligomerization formation in RBD and loss of function in ACE-2 thus, making binding, cellular receptor recognition impossible.

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General Significance

Ubrogepant represents a new therapeutic candidate in the fight against COVID-19, as it binds with relatively high affinity with free RBD, ACE-2 receptor and SARS-CoV-2 RBD/ACE-2 complex based on binding affinity calculations

Keywords: COVID-19; SARS-CoV-2 RBD; Ubrogepant; ACE-2; MD simulation

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Introduction

The SARS-CoV-2 is the causative agent of COVID-19; officially recognized as pandemic by WHO and affecting approximately 490,000 people and accounting for more than 22,000 deaths in more than 190 countries and territories of the world (Dong et al., 2020). The pathophysiology of COVID-19 is now emerging, so is the opportunity for drug development. The entire pathogenic episode is initiated by viral colonization of the nasal-trachea-pulmonary airway to pulmonary hyperinflammation (Liu et al., 2020), multiorgan failure and death (Lin et al., 2020).

Indeed, the adoption of interferon α (IFN-α, vapor inhalation), chloroquine, lopinavir/ritonavir and in the WHO guideline for treatment of COVID-19 were based on the clinical presentation of cases and pathological evaluation (Dong et al., 2020). Whilst the success of these therapeutic intervention has been subject of debates, the increasing number of SARS-CoV-2 proteins whose structures have been elucidated yet provided newer opportunities for new or repurposed drugs.

The main protease of SARS-CoV-2 co-crystallized with many inhibitors have been reported (Zhang et al., 2020); allowing the screening of novel candidates. Similarly, its spike glycoprotein (prefusion) with a single receptor-binding domain up (Wrapp et al., 2020); thus opening up opportunities for antibody and entry inhibitor design. The receptor binding domain (RBD) region of the surface spike glycoprotein (S protein) has been resolved in ACE2-bound state (Yan et al., 2020). Finally, the nucleocapsid protein N-terminal RNA binding domain has also been crystallized and currently been investigated as resource for drug development (Zhou et al., 2020).

Our group, is critically investigating RBD/ACE-2 complex for the purpose of drug and antibody repositioning. Over 3000 candidate compounds from U.S. Food and Drug

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Administration (FDA) database were evaluated for ability to bind RBD/ACE-2 interface; leading to the discovery of darunavir and ubrogepant (Omotuyi, 2020).

Whist darunavir is an anti-HIV drug acting via protease inhibition (Ghosh et al., 2007) but ubrogepant has no proven anti-viral activity. It is however worthy of note that ubrogepant is an oral, small-molecule calcitonin gene–related peptide receptor antagonist indicated for acute migraine treatment (Dodick et al., 2019), calcitonin gene–related peptide and its receptor interface is analogous to the RBD/ACE-2 interface under investigation.

Here, molecular dynamics simulation revealed that ubrogepant when bound to RBD/ACE-2 complex preferentially interacts with ACE-2 residues, and alters the dynamics of the complex.

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2. Materials and Methods

2.1. Starting structure:

The recently deposited SARS-CoV-2 spike glycoprotein RBD in ACE-2 bound state was used in this study (PDB ID: 6m17) all missing chains and broken residues were corrected using Swiss-PDBViewer suite. The structures of FDA library was retrieved from FDA webserver (https://www.fda.gov/drugs/drug-approvals-and-databases/drugsfda-data-files). Cheminformatic manipulation (removing all compounds with mass lesser than 200 and greater than 1700) of the library was performed using DataWarrior (Sander et al., 2015). Molecular docking runs were performed using mcule platform (https://mcule.com/). ubrogepant/ SARS-CoV-2-RBD complex was retrieved from mcule platform for molecular dynamics simulation.

2.2. Preparation of Complex for Molecular Dynamics Simulation

HTMD notebook was used to prepare all the complexes for dynamics studies using charmm36 forcefield. MD simulation for dynamics study was performed using ACEMD. The atomic parameters for ubrogepant were derived from CHARMM General Force Field (ParamChem) (Vanommeslaeghe et al., 2012). All conditions for equilibration and MD simulation have been previously reported by our group (Olaposi et al., 2019). The biosystems for Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) energy calculation (Kumari et al., 2014). were built using *charmm-gui* webserver (Jo et al., 2017) using charm forcefield from two conformations within the last 20 ns of the dynamics study. The simulation was performed using GROMACS (*ver.* 2018) (Kutzner et al., 2015), all procedure for minimization, equilibration and production have been previously reported (Omotuyi et al., 2015)

2.3 Post-MD analysis:

Network analysis was performed using network Tools program in-built in visual molecular dynamics (VMD) as we have previously reported (Omotuyi et al., 2015), GROMACS *in built* gmx distance tool was used to calculate interatomic/inter-residue distance. Water density Water density within the interface was also calculated using Volmap plugin in-built into VMD as we have previously reported.

Dynamical networks (set of nodes with connecting edges) for SARS-CoV-2 spike glycoprotein RBD in ACE-2 interface residues were calculated as described (Sethi et

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al., 2009) noting that the size of an edge corresponds to their weights. Unless otherwise stated, all line graphs were plotted as mean ± standard error of mean (S.E.M) from 2 ~ 3 independent runs using GraphPad prism (ver 6.0e, 2014).

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3.0 Results and Discussion

3.1 Molecular docking identifies ubrogepant as high affinity binder at the RBD/ACE-2 interface

Fusion or entry inhibitors are known to block the fusion of viruses to their cellular receptors, maraviroc and enfuvirtide are known examples indicated in HIV treatment (Qian et al., 2009). These agents specifically antagonize the interaction between viral surface glycoprotein receptor binding domain and the cellular receptor thereby blocking cellular entry. In case of SARS-CoV-2, the interaction between the cellular receptor ACE-2 and the viral glycoprotein has been well studied (Yan et al., 2020). When filtered compounds at the FDA database were docked into the interface, about three hundred compounds have energy values between *-10.9 and -5.0* kcal/mol (fig. 1A). One of the compounds was identified as ubrogepant (UBR, right panel, fig. 1A inset). A close look into the binding pocket revealed that a network of charged amino acid residues from RBD and ACE-2 populate the interface and play key roles in the estimated affinity. RBD contributes Q943, Y453, Y495, R 403 and Y505 to UBR binding while ACE-2 contributes K353, D38, E37, H34 and E35 (Fig. 1B).

At first glance, UBR is an orally available calcitonin gene-related peptide (CGRP) receptor antagonist without any known anti-viral potency, which may not present a likely candidate for the treatment of COVID-19, however, its mechanism of action as antagonist to peptide (CGRP)/receptor (CGRPR) interaction may present an opportunity for re-evaluation as RBD/ACE-2 is also peptide/receptor interaction. Secondly, the role of CGRP in the induction of eosinophil migration and the stimulation of beta-integrin-mediated T cell adhesion to fibronectin at the site of inflammation (Springer et al., 2003) is symptomatic of COVID-19 pathogenesis; thus, bringing UBR to focus as potential therapy in the current pandemic.

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3.2 Atomistic simulation identifies ACE-2 residues' preference for ubrogepant

Molecular docking may provide fast method for large chemical database screening of but it is usually poor in correctly estimating binding affinity and even poorer in predicting the stability of such compounds in the ligand pocket (Pantsar and Poso, 2018). In order to overcome these limitations, energy of binding, and the contribution of the residues to UBR binding were evaluated using Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) approach (Wang et al., 2017) atomistic simulation. In RBD/ACE-2 complex, RBD less (none of the residues contributed up to -10 kcal/mol) to UBR binding in comparison to ACE-2 . Specifically, D30, D38, D350 contributed significantly to UBR binding (Fig. 2A, *i&ii*, inset is the snapshot showing the Cα of the residues). When ACE-2 and RBD were tested for UBR binding separately, a nearly similar result (Fig. 2B, *i&ii*) was obtained strongly indicating that UBR binding is predominantly driven by ACE-2. The estimated energy of binding supports this conclusion as RBD/UBR, ACE-2/UBR and RBD/ACE-2/UBR binding energies were estimated as -45.93 ± 2.89, -50.19 ± 4.32 and -72.16 ± 4.09 kcal/mol (Table 1.0). By implication, UBR has the highest affinity for RBD/ACE-2 complex but can also bind to unoccupied receptor. This finding has significance in both prophylactic and curative course of treatment in clinical setting.

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3.3 Ubrogepant disrupts critical node of interaction at RBD/ACE-2 interface

From the current understanding from SARS-COV, molecular recognition at RBD and ACE-2 interface precedes virus binding/entry, membrane fusion and viral RNA release (Song et al., 2019). This implies that altering the molecular recognition pattern may prove to the effective at halting the downstream processes. Protein community network analysis was performed on RDB/ACE-2 complex with or without RBD following molecular dynamics simulations; this approach was used identify recognition/binding interactions and UBR effects. The overall result was presented as a dynamic contact map of the edges connected by nodes. Here, only the critical nodes were shown; representing the Cα of interacting residues positioned at optimal bond-forming distance (~4.0 Å) for at least 80% of the trajectories. Critical node, allow communication between different network communities (Sethi et al., 2009). In the absence of UBR, D30/L455, D38/Y449, K355/N501 and E329/R439 critical nodes were observed in ACE-2/RBD interface respectively (Fig. 3A, *i*). All the critical node-forming residues identified here are also within hydrogen-bound forming distance in the crystallized structure (Yan et al., 2020). In UBR-bound complex, most of the nodes have both been lost or/and altered (Fig. 3A, *ii*). Since trajectories along a MD simulation allows high resolution atomistic details, two key interface interactions were then studied. First, the crystal structure observed interaction between K31 and N493 and secondly, between E329 and R439 (ACE-2/RBD residues respectively). Interestingly, in the absence of UBR, K31/N493 fluctuated within hydrogen-bond forming distance (Fig. 3B, *i*) but not in the presence of UBR. Similarly, E329/R439 interaction was stably maintained in the presence of UBR but not in the apo complex (Fig. 3B, *ii*). Another key finding in this study is the hydration of the interface following UBR binding. In the crystal structure (PDB ID: 6m17), no water molecule was co-crystallized at the interface which is also reproduced by limited water occupancy in the apo system in this study (Fig. 3C, *i*); when UBR-bound biosystem was analyzed, the extended loop region was fully occupied

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with water (Fig. 3C, *ii*) which clearly underscores the suboptimal interaction between the interface residues. Clearly, these data strongly suggest that the dynamics of RBD/ACE-2 binding is alterable by UBR and predictably, altering the SAR_COV-2 binding and entry into host cells, UBR should completely block membrane fusion which is an essential step to viral RNA release.

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3.4 Ubrogepant-dependent fluctuations in RBD/ACE-2 complex

Atomic displacements in protein either reflected in the B factor values of the crystal structure or atomic fluctuations calculated along MD trajectories are indicative of protein motion and its dynamics which has direct correlation to protein function (Wall et al., 2014). In the foregoing, alteration in the localized region of the complex (RBD/ACE-2 interface) has been investigated, next, how binding of UBR at this local space affects the total conformational space of the protein was studied. Atomic fluctuation of ACE-2 residues alone was compared to RBD-bound and RBD/UBR complexes. Interestingly, two key features were noted; UBR- and RBD-dependent fluctuations. The zinc-binding site, spanning residues 350 to 425 exhibited UBR-dependent fluctuation (Fig 4A, *i*) but when it extends to residue 502 (active site), the presence of RBD is enough to increase its atomics fluctuation. Interestingly, the zinc-binding site is proximal to UBR binding site (Fig 4A, i, inset, UBR is shown in cyan sphere, the zinc-binding site is shown in red). Similarly, RBD residues exhibit atomic motions in ACE_2 dependent manner. At the RBD N-terminal (orange, Fig 4A, *ii*, inset), presence of ACE-2 increases atomic displacement; interestingly, this region has been documented as involved in spike glycoprotein oligomerization. For instance, murine hepatitis coronavirus forms dimers using a domain which overlaps its receptor-binding domain (Xiao et al., 2004). This dimeric state is central to receptor binding and cell entry. The results here strongly support that UBR may alter the dynamics of RDB with direct implication on RBD oligomerization, receptor recognition and binding.

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Conclusion

As the world battles against COVID-19, clinicians must depend on biomedical scientist to provide necessary therapeutic tools as effective drugs, vaccines or other forms of immunotherapy are key determinant of the case-to-fatality ratio in any pandemic.

Here, we have leveraged on the speed of molecular docking to screen likely candidates from database of FDA approved drugs. Two candidates have emerged darunavir (Omotuyi, 2020) and ubrogepant. From MD simulation and post-simulation data analyses, both candidates disrupt the interaction between RBD and ACE-2 receptor. Interestingly, we have revealed the details of this disruption. First, the being ruptures the interaction between the interface residues of the two proteins, followed by hydration of the interface which perpetuates the loss of interaction. Next, the RBD oligomerization interface and ACE-2 zinc binding site gain increased atomic fluctuation with direct implication on cellular entry. Finally, ubrogepant can bind free SARS-CoV-2 RBD, cellular membrane-bound ACE-2 and SARS-CoV-2 RBD/ACE-2 complex based on binding affinity calculations; thus making it clinically useful in all stages of infection.

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