JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2020.1850528

In silico screening predicts common cold drug Dextromethorphan along with Prednisolone and Dexamethasone can be effective against novel Coronavirus disease (COVID-19)

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Communicated by Ramaswamy H. Sarma

ABSTRACT

The ongoing outbreak of Coronavirus disease 2019 (COVID-19) is a matter of great concern. Although the mortality rate caused by this virus is less than that of SARS and MERS, it is showing higher efficacy in terms of human-to-human transmission. Several strategies have been taken by scientists and researchers worldwide to combat this virus. Numerous phytochemicals and synthesized chemicals are under incessant inspection to obtain a potent anti-covid drug. Since, till now no precise therapy is available for covid patients, researchers are trying to categorize all possible anti-covid substances. Repurposing of drugs and combined drug therapy are becoming popular in treating such viral diseases. In this study, we are proposing the repurposing of three chemicals-Dextromethorphan, Prednisolone and Dexamethasone as anti-covid agents. We have used the tertiary structure of Coronavirus main protease (M^{pro}) with PDB ID 6LU7 as the target protein in this analysis. Molecular docking and dynamics study further revealed their synergistic effect against the COVID-19 protease protein.

ARTICLE HISTORY
Received 20 August 2020
Accepted 6 November 2020

KEYWORDS
Covid-19;
Dextromethorphan;
Prednisolone;
Dexamethasone;
Coronavirus main protease (M^{pro})

1. Introduction

A novel acute respiratory Coronavirus disease 2019 (COVID-19) emerged in Wuhan, China in the fall of 2019 and was declared a pandemic by the World Health Organization (WHO) in March 2020. This is continuing its rampage over human life. The causative agent of this pandemic is Severe Acute Respiratory Syndrome–Corona Virus-2 (SARS-CoV-2). A huge number of people have been affected by this virus worldwide. Most patients showed high fever with loss of taste and smell with moderate respiratory problem, however, around approximately 5% of them developed severe respiratory distress and ultimately died (Guy et al., 2020). Regarding clinical features, COVID-19 is not very different from Severe Acute Respiratory Syndrome (SARS) and the Middle East respiratory syndrome (MERS). Moreover, the fatality rate of COVID-19 (2.3%) is significantly less than both SARS (9.5%) and MERS (34.4%) (Petrosillo et al., 2020). However, the major menace is the exponential transmission rate of SARS-CoV-2 that has recognized this virus as a global villain.

Coronavirus is a single-stranded RNA virus with a restrained vertebrate host range. The complete life cycle of this virus has several potentially targetable phases. For instance, (a) the endocytic entry of the virus into the host involving angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2), (b) RNA replication and transcription through helicase and RNA-dependent RNA polymerase (RdRp), (c) translation and processing of viral proteins via chymotrypsin-like and papain-like proteases, (d) assembly of the virion and (e) discharge of new viruses by exocytic systems. Targeting any one of these viral proteins may prove to be beneficial in the treatment process of COVID-19 (Abraham Peele et al., 2020; Guy et al., 2020). Unfortunately, the lack of medical supplements against this novel Coronavirus has made the scenario worst. Till now only supportive care is available to patients. Direct therapeutic care would aid the affected person in a better way. One approach to identify probable therapeutics against SARS-CoV-2 is repurposing already approved drugs against this deadly virus. This process takes the advantage of existing human pharmacology and toxicology knowledge and most importantly the chemicals/drugs are readily available for clinical trials (Elmezayen et al., 2020; Guy et al., 2020).

Studies on the effect of several potent phytochemicals against COVID-19 have been carried out recently (Aanouz et al., 2020; Abdelli et al., 2020; Adeoye et al., 2020; Al-Khafaji et al., 2020). Our group has also reported the effect of natural compounds from *Clerodendrum* as a possible therapeutic agent against COVID-19 (Kar et al., 2020). Previously, a combination of three therapeutic drugs lopinavir, oseltamivir and ritonavir has been reported to be effective in controlling the virulence of this virus among Covid patients within 48 h (Muralidharan et al., 2020). Reports are also there entailing the effects of steroids and senolytics against SARS-CoV-2 (Sargiacomo et al., 2020). A study on glucocorticoids as an anti-covid agent has been accounted but

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that work did not solely target the repurposing of those agents (Henderson et al., 2020). Here, we have considered Dextromethorphan (a constituent of Bromodex cough syrup), Prednisolone and Dexamethasone (exogenous glucocorticoids) for repurposing work against Covid and explored the synergistic mechanism of action among those chemicals. *In silico* docking and molecular dynamics (MD) simulation provided an insight into using a combination of these three compounds as potential therapeutics against covid. However, this is solely an *in silico*-based approach and is open for a clinical trial.

2. Materials and methods

2.1. Protein and ligand structure preparation

Structure of SARS-CoV-2 protease (PDB Id: 6LU7) was downloaded from the Protein data bank (PDB) (https://www.rcsb.org/). This structure has already been used as a target receptor protein for drug repurposing studies supporting its ideal candidature for this purpose. Three compounds namely Dextromethorphan (PubChem CID: 5360696) (a constituent of common cough syrup), Prednisolone (PubChem CID: 5755) and Dexamethasone (PubChem SID: 152235612) (exogenous glucocorticoid) were selected as our ligands and their structures were downloaded from NCBI PubChem database. Smiles server (https://cactus.nci.nih.gov/translate/) was exploited to convert SDF to PDB format. Those PDB structures were converted into pdbqt format after choosing torsion angles and identifying the rotatable bonds present within them. The receptor protein was prepared for docking after the deletion of water molecules and the addition of polar Hydrogen. Polar hydrogens were merged with non-polar hydrogen atoms. Gasteiger charges (–3.6) were assigned to the protein. Finally, the PDB format of the receptor was converted to pdbqt format and it was ready for docking.

2.2. Molecular docking

Both individual and sequential docking strategies were adapted for this study. The idea behind this strategy was straight-forward. Since we are going for a drug repurposing study, the ligand-binding sites may deviate from conventional binding sites of the protein where usually the anti-Covid drugs would bind. Hence, first, we performed a blind docking where the whole protein was taken inside the grid box and probable binding sites were searched against the three selected ligands individually. The best binding sites (BBS) for each ligand were marked. Following this, sequential docking was performed where we docked each ligand to their respective BBS in chronological conduct. Through this approach, we aimed to explore whether any synergistic or antagonistic effect exists among the considered ligands when they are present as one single complex. A very similar approach has been previously adopted by Muralidharan et al. (2020).

In this phase first, Dexamethasone was docked. Since few reports mentioned Dexamethasone as one of the possible anti-covid drugs (Patel et al., 2020) it was first docked to its identified BBS. We named the complex 6LU7-D1. Following this, Prednisolone, another FDA-approved well-acclaimed steroid (glucocorticoid) was docked with 6LU7-D1 and was named as 6LU7-D1-P. Next, Dextromethorphan was docked to 6LU7-D1-P and the final docked complex was named 6LU7-D1-P-D2.

2.3. Molecular dynamic approach

MD simulation has been widely used to examine atomic behavior, structural stability and conformational changes at the atomic level of a protein. The 6LU7-D1-P-D2 complex was subjected to MD simulation by Gromacs (Pronk et al., 2013) using the Gromacs96 53a6 force field. The topology files were generated in GROMACS software. The macroscopic variables can be defined as quantities influencing the microstates of a reaction such as number of particles in the system (N), the system's volume (V) and total energy in the system (E). Altogether this is known as NVE ensemble (Dunkel & Hilbert, 2014). We must introduce Thermostat and Barostat for running MD simulation at NVE system (http://klingon.uab.es/prat/Thesis/node46.html). For doing this, we have to take a constant temperature and constant pressure. The temperature and pressure were set to 303 K and 1 bar, respectively. Constant pressure and temperature were set for equilibration steps. This process was adopted from Khan et al. (2020). MD simulations were performed for a 100 ns time scale, with 10,000 steps of energy minimization through the steepest descent mechanism. MD trajectories were estimated through the root-mean-square deviation (RMSD), as well as the root mean square fluctuation (RMSF) of the complexes.

3. Result and discussion

3.1. Synergy of selected drugs against SARS-CoV-2 protease

Both individual and sequential docking approaches were adapted for this study. The individual docking scores for Dexamethasone, Prednisolone and Dextromethorphan were −8.4 kcal/mol, −8.2 kcal/mol and −7.9 kcal/mol. BBS for Dexamethasone was found to be on the A chain of 6LU7 protein. Arg105 (A), Ile 106 (A), Gln110 (A), Asn151 (A), Phe294 (A), Asp153 (A), Ile 152(A) were the interacting amino acids. Prednisolone best interacted with Arg298 (A), Tyr154 (A), Ile152 (A), Asp153 (A), Phe8 (A), Pro9 (A) and Lys12 (A). Tyr239 (A), Tyr237 (A), Leu272 (A), Gly275 (A) and Leu287 (A) were present in the BBS of Dextromethorphan. Overall the individual dock scores were considerable for clinical repurposing trials with each selected ligand.

Now, the most interesting question was if these three compounds are used in combination will they show a synergistic effect or antagonistic effect? There are several reports of successful combinatorial drug therapies (CDT) especially against the virus, for instance, HIV. Since both COVID and HIV are single-stranded-RNA viruses and CDT has proved to be a huge success against HIV, there lies a colossal probability of CDT success against SARS-CoV-2. Interestingly in our study, the docking score of Prednisolone increased to −8.6 kcal/mol (from

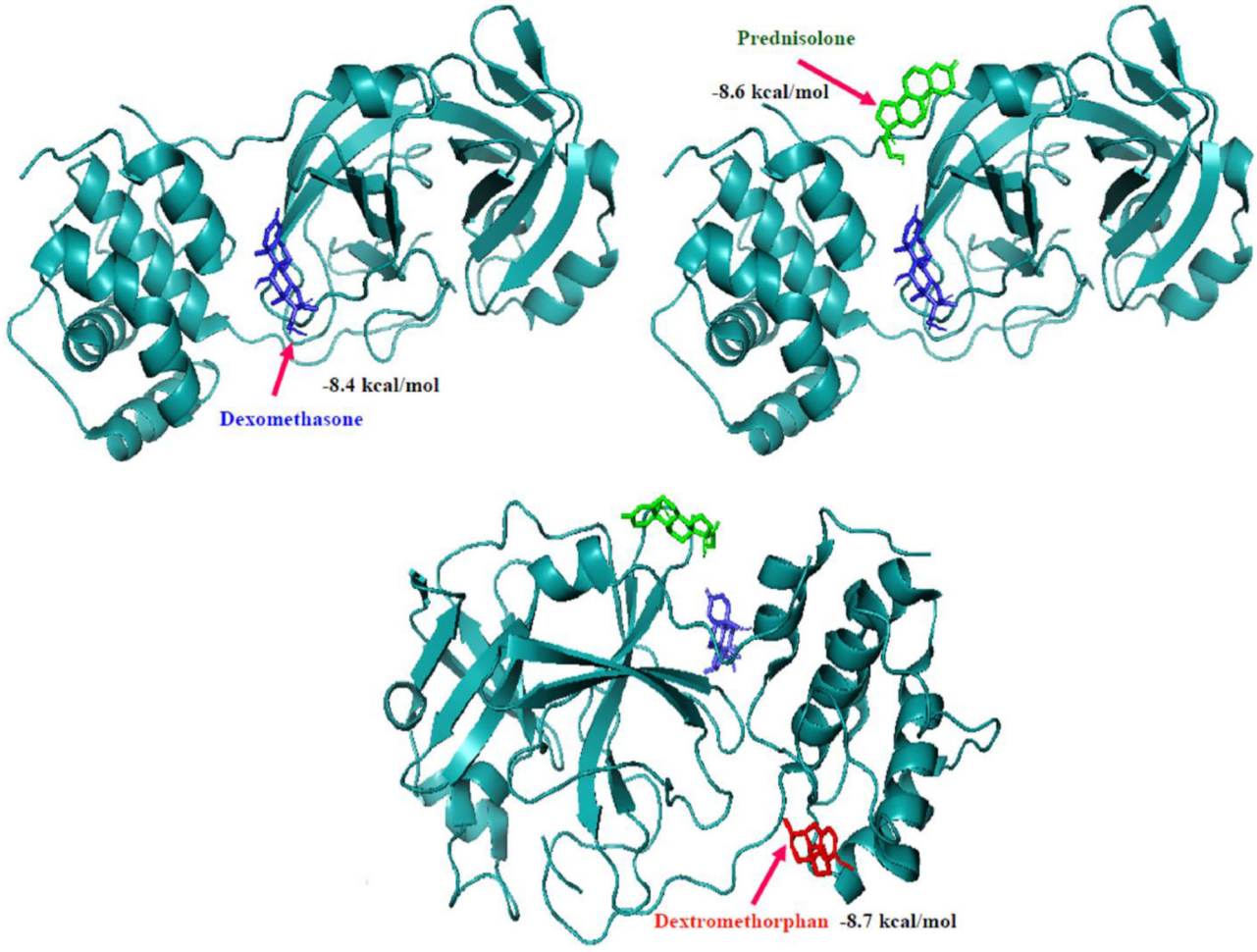

Figure 1. Synergistic effects among selected ligands evident from sequential docking study. Different color codes have been used for different ligands and have been mentioned within the figure.

−8.2 kcal/mol) if Dexamethasone was already present with the protein structure (6LU7-D1) and Dextromethorphan showed −8.7 kcal/mol (from −7.9 kcal/mol) binding energy if both Dexamethasone and Prednisolone were complexed with the target protein (6LU7-D1-P complex; Figure 1). This result clearly showed the synergistic effect of considered ligands indicating that a combination of these drugs is more effective against the COVID-19 protease enzyme rather than their individual usage.

Another aspect we explored is the effect of these drugs on the primary immune response of cytokine production. Body's early immune response produces large amounts of cytokines to ward off infecting agents. Cytokines are proteins associated mainly with inflammatory response causing fever and inflammation. This 'cytokine storm' leading to excessive inflammation is believed to cause severe COVID-19 symptoms leading to death (Henderson et al., 2020). Life-threatening lung-damage and inflammation have been reported in critically and severely ill Covid patients. Dexamethasone and Prednisolone, two well-known exogenous glucocorticoids with their anti-inflammatory and immunomodulating properties may thus be able to ramp down the excessive COVID-19 symptoms (Favalli et al., 2020; Shabalin et al., 2020).

On another note, Dextromethorphan has antitussive activity and is devoid of analgesic or addictive property. Moreover, this can cross the blood-brain barrier to activate the sigma opioid receptors present on the cough center of the central nervous system and suppress the cough reflex (Bem & Peck, 1992). This antitussive effect of Dextromethorphanis generally mediated via sigma receptors which inhibit the citric acid-induced cough reflex (Brown et al., 2004). Cough is one of the major symptoms of COVID-19, and thus, can be treated safely with Dextromethorphan (Figure 2).

3.2. Root-mean-square deviation

To investigate the changes in the molecular dynamics of protein along with the conformational stability of the protein–ligand complex, the RMSD value of the single protein (6LU7) and ligand–docked protein (6LU7-D1-P-D2) were compared.

The RMSD values of C-alpha atoms were plotted against time. RMSD for 6LU7 varied approximately from 1.5 to 3.1 whereas the value ranged from 1.0 to 2.1 in 6LU7-D1-P-D2 (Figure 3(a)). The C-alpha atom of 6LU7-D1-P-D2 showed slight fluctuation at 32–35 ns and 65 ns and remained stable for the rest of the time. A lower RMSD value of 6LU7-D1-P-D2 than 6LU7 indicated the conformational stability of the protein–ligand complex. This further strengthens our selected ligands as potential therapeutic agents against COVID-19.

3.3. Root mean square fluctuation

RMSF plot popularly depicts those residues which have experienced major fluctuations during the MD simulation process (Muralidharan et al., 2020). RMSF was measured for C-alpha atoms of each amino acid and was plotted against the number of residues. This plot revealed an identical pattern of residue fluctuation profiling for both 6LU7 and 6LU7-D1-P-D2 (Figure 3(b)). Conformational stability of a protein–ligand complex can be inferred if no considerable changes can be seen before and after MD simulations in the interaction patterns of a complex (Khan et al., 2020). In this study, 100 ns MD simulation of the 6LU7-D1-P-D2 complex revealed no major changes in their binding pattern. RMSF analysis indicated that the simultaneous binding of three selected ligands to the Covid protease raised no major complications in terms of protein flexibility and structural conformations thus reinforcing the idea of CDT with investigated ligands.

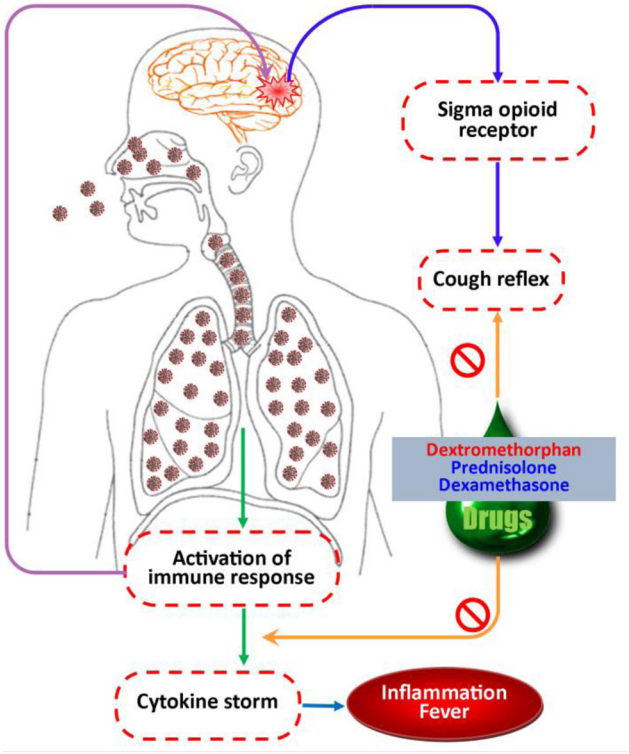

Figure 2. Possible mechanism of action of three ligands considered for this study as anti-covid therapy.

4. Conclusions

COVID-19, an evolving real-time global pandemic caused by SARS-CoV-2 has already killed more than 7.5 lakhs people worldwide and around 2 crores are affected (according to https://www.worldometers.info/Coronavirus/ 13 August 2020, 11.48 pm). The global socio-economic base has been thoroughly quivered by this tiny little virus. Several ideas have been put forward to combat the COVID-19 effect, however; there is still only supportive care available to patients. In this scenario, we undertook a repurposing project of common cough and cold drugs – Dextromethorphan, Prednisolone and Dexamethasone to explore their anti-Covid property. Individual and sequential docking study with MD simulation, RMSD and RMSF analysis revealed a combination of these three considered ligands may prove to be a successful therapy against COVID-19. However, this investigation is completely based on the *in silico* approach and is open for clinical trials to further evaluate the potency of the selected ligands in Covid treatment.

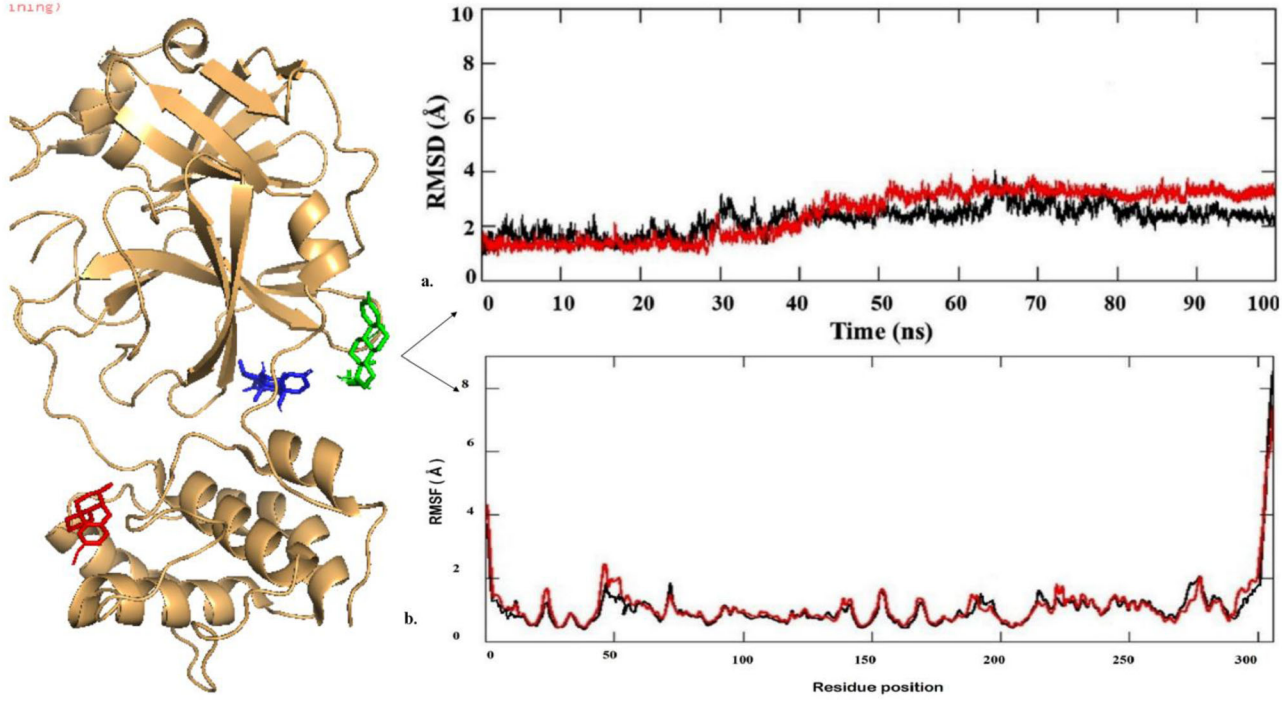

Figure 3. (a) RMSD plot of 6LU7 and 6LU7-D1-P-D2 complex. Red and black colors indicate 6LU7 and 6LU7-D1-P-D2, respectively. (b) RMSF plot of 6LU7 and 6LU7-D1-P-D2 complex. Red and black colors indicate 6LU7 and 6LU7-D1-P-D2, respectively.

Acknowledgements

We thank the University of North Bengal, Department of Biotechnology, Govt. of India and Department of Biotechnology, Govt. of West Bengal. Thanks are also due to Gargi Sen for her help in docking experiments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work is partially funded by the Department of Biotechnology, Govt. of West Bengal.

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