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In Silico studies of Natural compounds that inhibit SARS-CoV-2 Nucleocapsid Nsp1/Nsp3 proteins mediated Viral Replication and Pathogenesis

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Research Article

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Abstract

Highly Transmissible and pathogenic coronavirus that emerged in late December of 2019 caused Severe acute respiratory syndrome (SARS-CoV-2), which challenged human health and public safety. Severity of the disease depends on the viral load and the type of mutation that occurred in the coronavirus. Nonstructural proteins like, Nsp1, Nsp3, Nsp12 and Nsp13 including other viral proteins plays important role during viral replication life cycle. Viral Replication initiated by hacking the host cellular mechanism either by synergy or by suppression using nucleocapsid proteins of the virus. Spike (S) protein of the SARS-CoV-2 uses angiotensin-converting enzyme II (ACE2) and TRMPSS as a cell entry. Once virus enters host cell, nucleocapsid proteins along with its genome is releases from endosomes into cytosol of the host cell. Ca^{2+}/CaM (Calmodulin)/Calcineurin complex of the host cell plays important role during viral replication which is mediated by nucleocapsid proteins of the virus. Nsp1/Nsp3 nonstructural proteins triggers synergetic activity with CD147/CyPA/HSPG pathway and TRMP2/ADPr/Ca^{+2} mediated Ca^{2+}/CaM (Calmodulin)/Calcineurin synthesis and free radicle generation in mitochondria leading to viral replication and severe chemokine activation pathways. Docking studies were carried out to inhibit Cyclophilin A and TRMP2 proteins as drug targets. Natural compounds like Withanolide A, Columbin, Cucurbitacin E, Boswellic acid along with Cyclosporines, Vitamin E and N-Acetyl cysteine (NAC) were selected as ligands to study docking studies. Withanolide A and Cyclosporines had shown good inhibition activity against Cyclophilin A, whereas Columbin, Boswellic acid, Cucurbitacin E, Vitamin E and N-Acetyl cysteine (NAC) had shown inhibitory activity against TRMP2. Thus, we suggest conducting further studies to conclude above pathways mechanism and inhibitory effect of natural compounds against the Nsp1/Nsp3 mediated pathways Invitro and In vivo.

Introduction

SARS-CoV-2 Nucleocapsid proteins activation of host cellular responses

SARS-CoV-2, COVID-19 causing coronavirus caused pandemic and global economic instability bringing uncertainty. From the day of pandemic outbreak scientists are trying to understand the mechanism of the virus and its pathogenicity. Severity of the disease depends on its viral load and mutation present in Nsp1 and Nsp3 nucleocapsid proteins of the coronavirus. SARS-CoV-2 spreads from human to human through respiratory droplets by air and surfaces as medium. Spike (S) protein of SARS-CoV-2 uses angiotensin-converting enzyme II (ACE2) and TRMPSS as a cell entry. Nonstructural proteins like Nsp1 and Nsp3 work on crosstalk mechanism activates viral replication. Nsp3 activates TRMP2 leading to Calcium^{2+} cytosol influx and Potassium (k^{+}) ions outflux leading to cellular depolarization. Depolarization leads to ROS, NOX free radicle production in mitochondria. TRMP2 possess both ion channel and ADP-ribose hydrolase activity. ADP-ribose accumulation enhanced by free radicles and cellular depolarization leading production of IL-6, IL-10,TNFa and IL-8. Increase in Free Radicle production leads to Cyclophilin A (CyPA) synthesis. Nsp1 activates CyPA and facilitates Heparin sulfate proteoglycan (HSPG) binding which leads

to CD147 expression. This entire process leads to Chemotaxis and leukocyte activation. CyPA/HSPG complex mediates Ca $^{2+}$/CaM (Calmodulin)/Calcineurin pathway of producing IL-2 cytokines through NFAT (Nuclear factor of activated T-cells). Both Nsp1 and Nsp3 mediated pathways by crosstalk mechanism facilitate Viral Replication through Ca $^{2+}$/CaM (Calmodulin).We suggest researchers to further study Nsp1 mediated activation of CD147/CyPA/HSPG pathway, Nsp3 mediated activation of TRMP2/ADPR/Ca^{+2} pathways integrated with calmodulin enhance SARS-CoV-2 Viral Replication and chemokine storm. Earlier research had proposed TRMP2(Transient receptor potential melastatin 2) over expression leading to neurological disorders and also loss of smell because of Zn^{2+} endosomal cytosol toxicity.

TRMP2 Calcium influx mechanism

ADP-ribose (ADPR) mediated gating of TRP (melastatin) 2 (TRPM2) is a unique functional property of the transient receptor potential (TRP) family of cation channels. ADPR binds to intracellular C-terminal tail of TRPM2 with pyrophosphatase activity. TRMP2 gated ADPR enhances Cytosolic Ca^{2+} influx. NAD and H(2)O(2) and cyclic ADPR are other pathways of TRMP2 activity that may act synergistically. H(2)O(2) mediated oxidative stress may also induce formation of ADPR in the mitochondria or nuclei. In Oxidative stress mediated cell death TRPM2 is likely to be a key player and its activation leading to mitochondrial damage. In vitro Research had shown additional supplementation of calcium had inhibited TRMP2 activity.

CD147/CyPA/HSPG mediated chemotaxis

CD147 widely expressed plasma membrane protein implicated in variety of pathological and physiological pathways has ability to function as extracellular matrix metalloproteinase inducer which regulates lymphocyte responses. Cyclophilins are the interacting proteins with CD-147 leading to structural and medical implications. Chemotactic activity of Cyclophilins A and B mediated by CD-147 as signaling receptor towards a variety of immune cells. Recent studies demonstrated that cyclophilin–CD147 interactions in the regulation of inflammatory responses in acute lung inflammation, rheumatoid arthritis and cardiovascular disease. Heparan sulphate proteoglycans (HSPGs) plays important role in both the signaling and chemotactic activities of CyPA and CyPB and also as their primary binding site on target cells. HSPGs knockout from the cell surface of neutrophils can eliminate signaling responses to cyclophilins and inhibit cyclophilin dependent chemotaxis and adhesion of neutrophils and T cells.

Methods

Crystal structures of the protein-ligand complexes used in this study obtained from Databank http://www.rcsb.org/pdb. Ligand site was carefully checked when asymmetric unit found different from

biological unit. The biological unit used in this study are nucleocapsid proteins NSP1and NSP3 of SARS-CoV-2 with PDB ID: 7K3N, 6YWM and Host cellular proteins TRMP2, Cyclophilin A with PDB ID: 6MJ2,1FGL. From PDB Database Experimental protein-ligand binding affinities were taken. From recent publications protein ligand PDB core set was taken. The following criteria were taken for the structures to run docking: Crystallographic resolution lower than 3.2 Å non-covalent binding between protein and ligand. Structurally diverse ligands in complex with a heterogeneous collection of proteins Derived from the complex crystal structure flexible docking of the ligand to rigid receptor carried out. Two different input methods were used to prepare the structures: (a), using Gasteiger charges for both the ligands and the proteins; (b) PM6 charges were calculated by MOPAC2009 for both the ligands and proteins. For the input structures Gasteiger charges were prepared as follows: Hydrogen atoms were added using AutoDock tools and ligand atoms and bond types were assigned.

Gasteiger charges were used to calculate Empirical charges. HEME and metal ions were kept for proteins and cofactors, and their atom types and bond types were assigned manually. Halogen, Sulphate atoms and water molecules were removed. Hydrogens were added in protein residues along with Gasteiger partial charges using AutoDock tools. Sulphate, halogens and water molecules were removed. Non-polar hydrogen atoms along with their charges were merged and Hydrogens are added in protein residues as well as Gasteiger partial charges using Auto Dock Tools. No further optimization of the protein structures was done. PM6 method was used to assign semi-empirical modules by Mozyme function of MOPAC2009 program in Docking Server http://www,dockingserver.com. In the last step PM6 charges were calculated using MOPAC2009 software for the Ligand structures with semi-empirical charges and followed as above description. Halogens, Sulphate and water molecules were removed first for the Protein structures setup. AutoDock Tools were used to add Hydrogen atoms to the pdb structures. Mozyme functions of MOPAC2009 software was used to calculate the total charge of the protein and partial charges of the atoms. Further the calculated partial charges were applied. Compounds like Withanolide A, Columbin, Boswellic acid, Cucurbitacin E and Cyclosporines , Vitamin E and N-acetyl cysteine are selected as ligands and Docking studies were performed using Docking Server http://www.dockingserver.com. In order to calculate partial charges while ligand setting Docking Server integrates Marvin http://www.chemaxon.com and MOPAC2009 at a given protonation state and for semi-empirical geometry optimization. For docking calculation, AutoDock 4 is integrated. PM6 method, QASP parameter modified (QASP = 0.00679) both Autogrid 4 and AutoDock 4 were used in case where protein and ligand partial charges were calculated.

Results

Docking results and some existing research studies had shown Compounds like Withanolide A, Columbin and some Repurposed antiviral drugs proven binding efficacy to Spike protein and inhibit viral replication. Anti-inflammatory and immune suppressive drugs like Cyclosporines, Withanolide A, Boswellic acid had inhibitory effect on CD147/CyPA/HSPG pathway thus reducing viral amplification and IL-2 production.

Natural compounds like Vitamin E, N-Acetyl Cysteine (NAC), Columbin and Cucurbitacin E had shown inhibitory effect against TRMP2/ADPR/Ca^{+2} pathway thus reducing IL-8/IL-6/IL-10 and TNFa production see Table 1and Figure 2. We are proposing the pathway in Figure-1 based on our gene ontology studies and previous research data published.

Note

Authors has no conflict of interest

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Tables

Table 1: Docking results of Compounds against target proteins

S.No	Compound	Protein target	Estimated free energy Kcal/mol,DG
1.	Withanolide A	7K3N(NSP1),6YWM(NSP3),6MJ2(TRMP2),1FGL	-5.45,-7.99,-8.01, -7.12
I2.	Columbin	7K3N(NSP1),6YWM(NSP3),6MJ2(TRMP2), 1FGL	-5.67,-8.2,-6.25, -4.97
3.	Cucurbitacin E	7K3N(NSP1),6YWM(NSP3),6MJ2(TRMP2), 1FGL	-5.89,-6.75,-6.9, -4.92
4.	Boswellic acid	7K3N(NSP1),6YWM(NSP3),6MJ2(TRMP2), 1FGL	-5.24,-5.76,-7.16, -4.58
5.	Vitamin E	6MJ2(TRMP2), 1FGL	-5.23, -6.77
6.	Cyclosporine	6MJ2(TRMP2), 1FGL	-5.12, -6.95
7.	N-Acetyl Cysteine	6MJ2(TRMP2), 1FGL	-5.54, -7.12
8.	Loliolide	7K3N(NSP1),6YWM(NSP3),6MJ2(TRMP2), 1FGL	-5.34,-6.32,-7.56, -6.75
9.	Abscisic acid	6MJ2(TRMP2), 1FGL	-5.96,-6.22

Figures

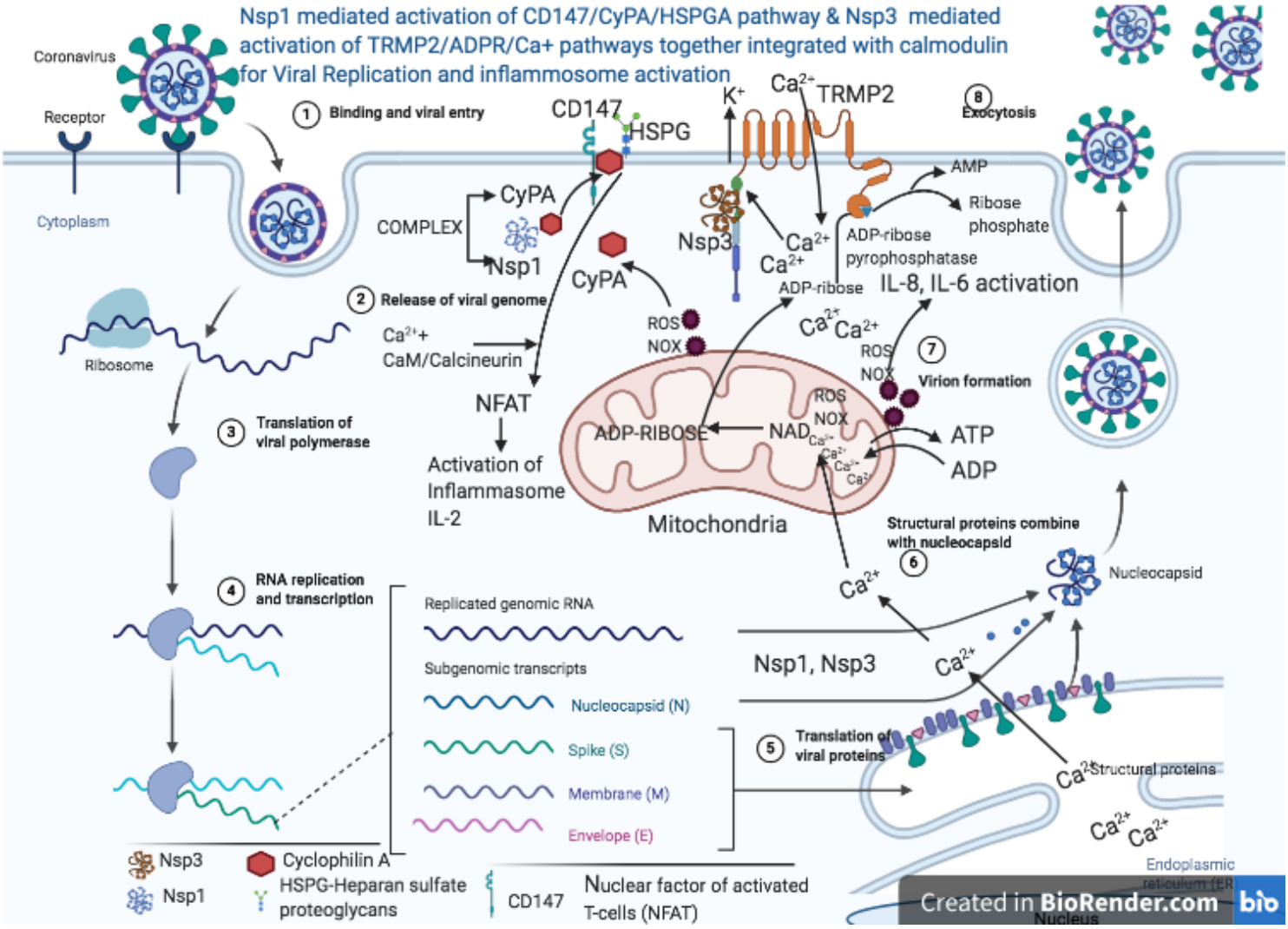

Figure 1

proposed pathway, Nsp1 mediated activation of CD147/CyPA/HSPG pathway, Nsp3 mediated activation of TRMP2/ADPR/Ca+2 pathways integrated with calmodulin enhance SARS-CoV-2 Viral Replication and chemokine storm

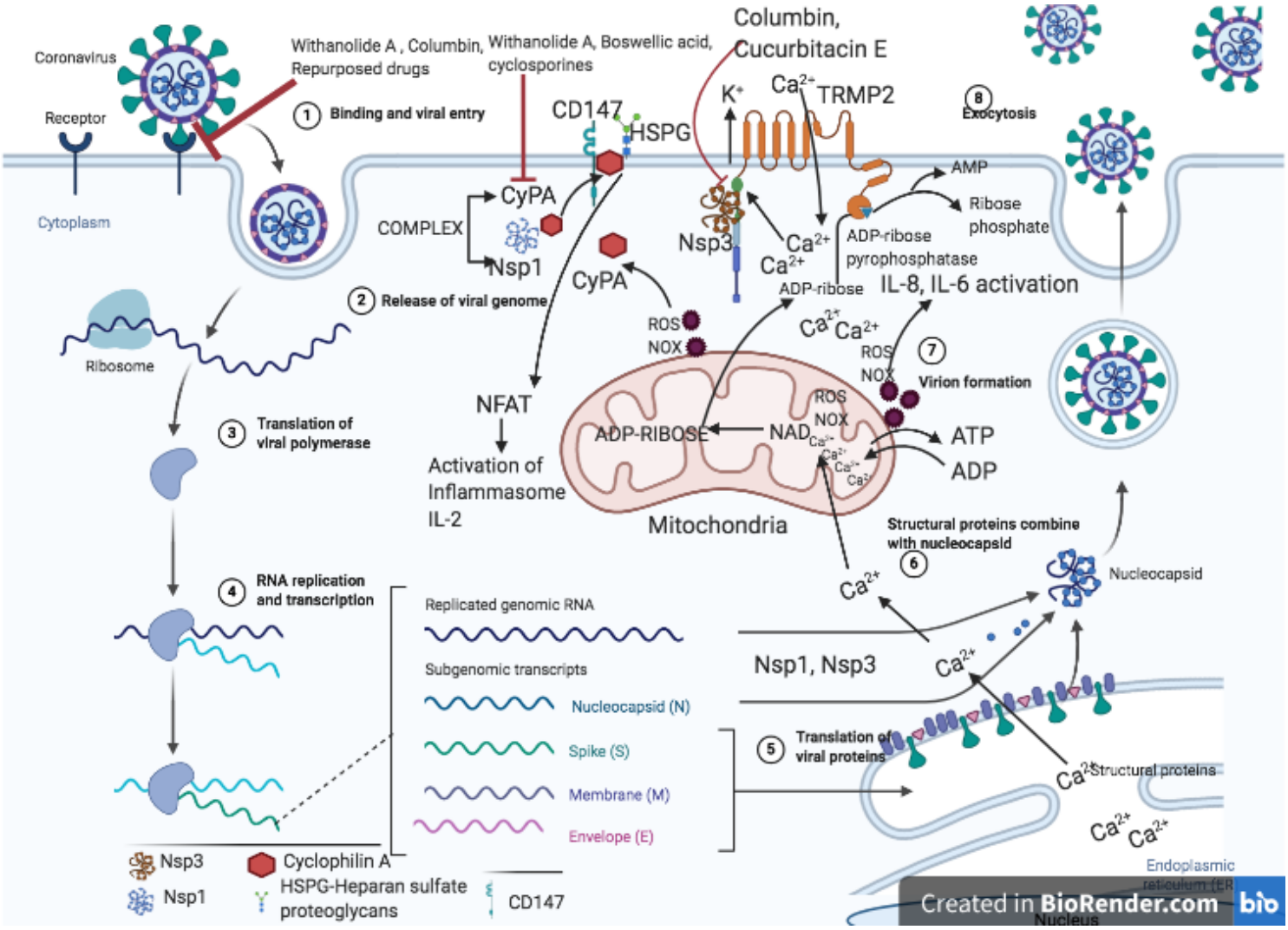

Figure 2

Proposed molecules to inhibit Nsp1 mediated activation of CD147/CyPA/HSPG pathway, Nsp3 mediated activation of TRMP2/ADPR/Ca+2 pathways integrated with calmodulin.