

Potential antiviral mechanism of hydroxychloroquine in COVID-19 and further extrapolation to celecoxib (Celebrex) for future clinical trials.

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Abstract

We are proposing a new hypothesis for the antiviral mechanism of hydroxychloroquine (HCQ), based on our computer aided docking studies. HCQ is a clinical trial drug under investigation for the treatment of Covid-19 pandemic. SARS-CoV-2 is the causative agent of the Covid-19 and it binds to host's bromodomain proteins BRD-2/4. The bromodomain proteins are readers of acetylated histones and play a critical role for host's hyper-immune response to this pathogen. Covid-19 virus protein E mimics acetylated histones and binds at the same site on BRD-2. We propose that the hijacking of BRD-2/4 by SARS-CoV-2, can be thwarted by the use of inhibitors of BRD-2/4. Preliminary *in-silico* docking studies with HCQ, indicates that it binds to the same pocket on BRD-2 where viral envelope protein E binds. Therefore, HCQ may act as a BRD-2 inhibitor, thereby preventing "cytokine storm" initiated by SARS-CoV-2 virus. Another FDA approved drug celecoxib (Celebrex) binds in the same pocket of BRD-2 and the key amino acid interactions between the drug and protein are conserved. Thus, celecoxib may offer innovative path for controlling the Covid-19 pandemic.

The immune system response is important for fighting the pathogen attack, however it often leads to deterioration of host body due to overproduction of inflammatory proteins. The activation of inflammatory gene expression is the hallmark of host-immune system pathogen interactions (1). The post-translational modification of histones by nuclear proteins is critical for inflammatory gene expression (2). One such modification is acetylation of lysine residue on histones which triggers the production of cytokines. The family of proteins called as bromodomain and extra terminal domain (BET) play important role for recognizing acetylated lysine residues on histone. BET protein family are "readers" of histone acetylation and play critical role in gene transcription (3).

SARS-CoV-2 responsible for the Covid-19 pandemic, hijacks the process of recognition of acetylated histones by BET proteins and disrupts the post-translational modification of histones (4). SARS-CoV-2 envelope transmembrane protein E binds to two important members of BET: BRD-2 and BRD-4 (4). The protein E mimics the acetylated histone where binding of BRD-2 occurs, thereby changes the host's protein expressions in favor of viral survival. Therefore, targeting BRD-2, which is the key to host's hyper-immune-response can be potentially useful strategy for fighting "cytokine storm" initiated by SARS-CoV-2, which ultimately proves fatal in many cases (5).

Recently hydroxychloroquine (HCQ) has shown promise in fighting Covid pandemic in clinical trial in china (6,7). We are proposing BRD-2 and BRD-4 as potential targets for the anti-SARS-CoV-2 action of HCQ. We used previously published crystal structures of BRD-2 (PDB: 5UEW) (8) to perform *in silico* docking studies using Autodock 4.2 (Scripps Research Institute) for HCQ and other FDA approved drugs. Our preliminary

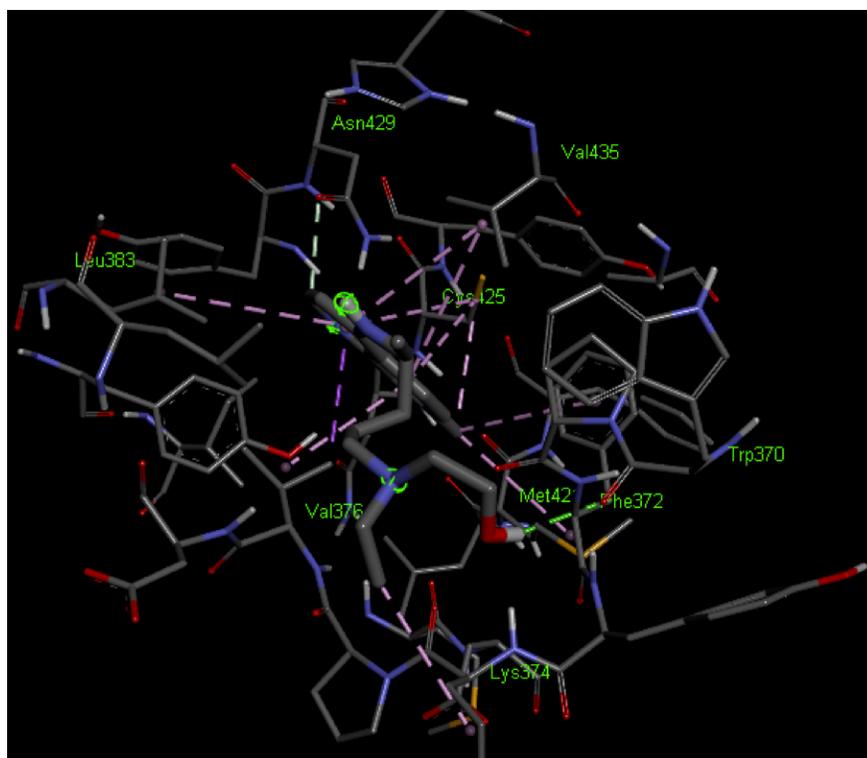
results indicated that HCQ binds exactly at the same BRD-2 site, where acetylated lysine residue of the histone binds, therefore our hypothesis is that HCQ thwarts the hijacking of BRD-2 by SARS-Cov-2. HCQ binding interactions involve critical amino acid residues of BRD-2 required for the recognition of histone namely: 1) Asparagine (Asn 429), which is conserved in all isoforms of BET and is the key residue binding to acetylated lysine of histone 2) Valine (Val 435) which is the “gatekeeper” of binding pocket 3) WPF shelf residues (Trp 370). These amino acid residues are also conserved in BRD-4 (2,3,8). Therefore, there is high probability that the HCQ may acts as the inhibitor of BRD-2 and BRD-4, which explains the mechanism of action of HCQ against SARS-CoV-2. The same binding pocket is occupied by other BRD-2 inhibitors and similar drug-receptor interactions are observed with small molecules such as JQ-1, RVX-OH and NSC127133 (9,10,11). However, all these inhibitors are either in preclinical trial or not tested in humans. Given the scale of Covid pandemic and urgency to find the molecules to fight this pandemic we wanted to see if any FDA-approved drugs can bind to the same BRD-2 pocket where HCQ and JQ-1 are binding and engaging in important amino acid residues as listed above.

Our preliminary studies indicated that anti-inflammatory drug celecoxib (Celebrex) not only binds to the same pocket as previously described BRD-2 inhibitors, but also important drug-protein interactions are conserved as seen in the figures below. Therefore, we strongly believe that celecoxib can inhibit BRD-2 and thereby serve as important drug for fighting the Covid-19 pandemic. We agree that that this is a very preliminary and highly speculative hypothesis and we do not know all the aspects of this proposed mechanism. The computational method we used is relatively simple rigid protein docking and by no means is rigorous mathematical work. However, we hope that somebody with enough resources and access to SARS-CoV-2 can check the validity of this hypothesis, which may pave a way to do clinical trial for use of celecoxib (Celebrex) in Covid-19.

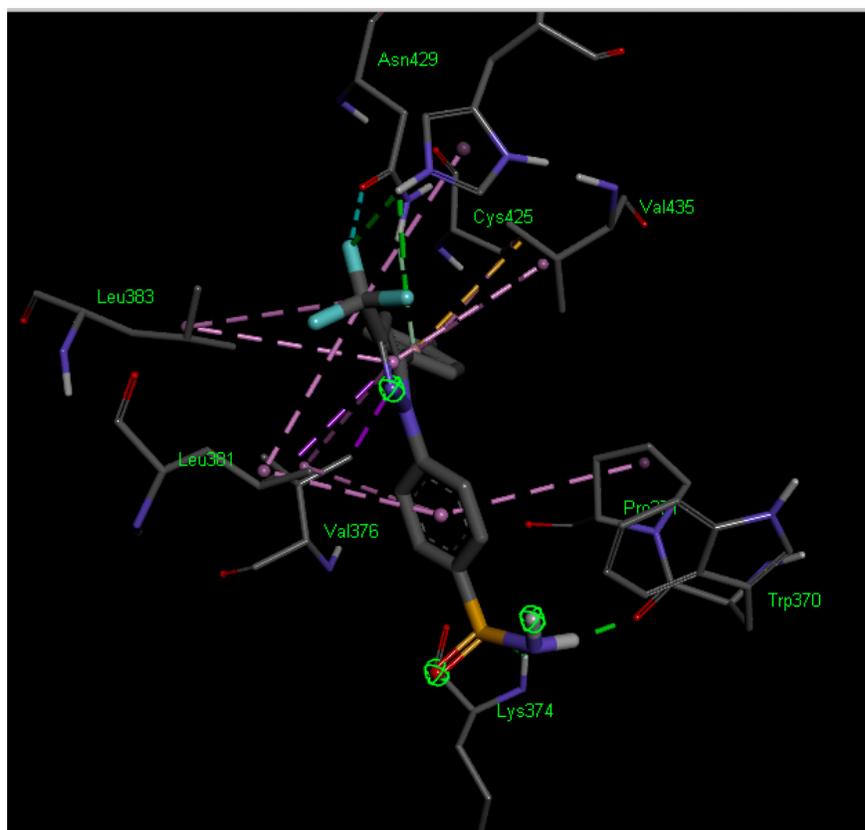
Figures:

Binding interactions of inhibitors involve important amino acid residues of BRD-2 namely: 1) Asparagine (Asn 429), which is conserved in all isoforms of BET and is the key residue binding to acetylated lysine of histones 2) Valine (Val 435) which is the “gatekeeper” of the binding pocket 3) WPF shelf residues (Trp 370, Pro 371, Phe 372).

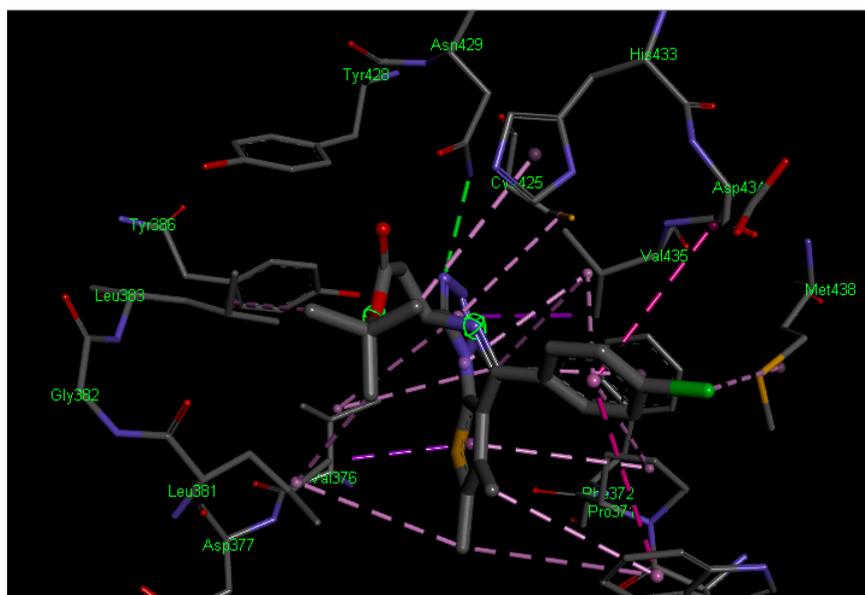
A) A) Binding interactions of Hydroxychloroquine (HCQ) with BRD-2



Binding interactions of Celcoxib (CLXB) with BRD-2



Binding interactions of JQ1 with BRD-2



Comparison of Bonding interaction of HCQ, CLXB and JQ1 with BRD-2

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