

SHORT COMMUNICATION



Computational and experimental insights on the interaction of artemisinin, dihydroartemisinin and chloroquine with SARS-CoV-2 spike protein receptor-binding domain (RBD)

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ABSTRACT

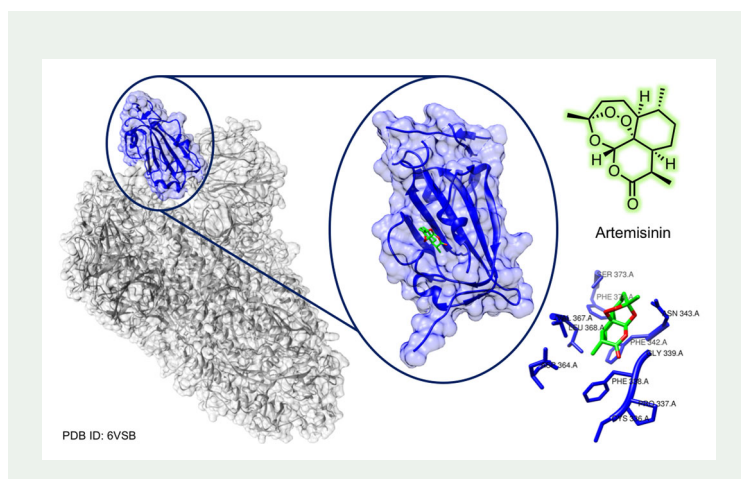
The mechanism of host cell invasion of severe acute respiratory syndrome coronavirus-2 SARS-CoV-2 is connected with the interaction of spike protein (S) with angiotensin-converting enzyme 2 (ACE2) through receptor-binding domain (RBD). Small molecules targeting this assembly are being investigated as drug candidates to contrast SARS-CoV-2. In this context, chloroquine, an antimalarial agent proposed as a repurposed drug to treat coronavirus disease-19 (COVID-19), was hypothesized to bind RBD among its other mechanisms. Similarly, artemisinin and its derivatives are being studied as potential antiviral agents. In this work, we investigated the interaction of artemisinin, its metabolite dihydroartemisinin and chloroquine with RBD by means of computational tools and *in vitro*. Docking studies showed that the compounds interfere with the same region of the protein and molecular dynamics (MD) simulations demonstrated the stability of the predicted complexes. Bio-layer interferometry showed that chloroquine dose-dependently binds RBD ($KD = 35.9 \mu M$) more efficiently than artemisinins.

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1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike protein (S) mediates cellular invasion by interfering with angiotensin-converting enzyme 2 (ACE2) through its receptor-binding domain (RBD, residues 331-524 of S1 subunit, [Figure 1A](#)) ([Tai et al. 2020](#); [Wrapp et al. 2020](#)). Targeting this assembly with small molecules, peptides and antibodies emerged as a possible intervention strategy to contrast infection ([Al Adem et al. 2020](#); [Xiu et al. 2020](#); [Chen et al. 2021](#)). The antimalarial agent chloroquine is thought to be active against SARS-CoV-2 through a combination of mechanisms, such as the inhibition endocytic pathways by elevation of endosomal pH and the interference with glycosylation of ACE2 ([Vincent et al. 2005](#); [Mauthe et al. 2018](#); [Gendrot et al. 2020](#)). Moreover, computational evidences suggested that chloroquine could also directly interfere with S or ACE2 proteins ([Fantini et al. 2020](#); [Badraoui et al. 2021](#)). The efficacy of chloroquine is a debated issue. Nevertheless, it can block the binding of SARS-CoV-2 to ACE2 *in vitro* ([Wang et al. 2020](#)). On similar basis, other anti-malarial agents such as artemisinins demonstrated *in vitro* inhibition of SARS-CoV-2 ([Krishna et al. 2021](#); [Kavak et al. 2021](#)). Extracts from the sweet woodworm *Artemisia annua* have been used for centuries in Chinese traditional medicine for treating the symptoms of febrile diseases, tidal fever and summer heat stroke ([Brown 2010](#)). In particular, a compound isolated from this plant and known as “qinghaosu”, which was later renamed artemisinin, was tested in patients together with its derivatives between 1970s and 1980s for its activity against malaria ([Aweeka and German 2008](#)). From a chemical point of view, artemisinin is a sesquiterpene lactone containing a peroxide bridge ([Nahar et al. 2020](#)). Derivatives such as artesunate, artemether and arteether have been used in clinic against malaria for decades ([Aweeka and German 2008](#)). Moreover, artemisinins are being investigated to treat diseases such as cancer, inflammatory conditions, immunosuppression and viral or fungal infections ([Ho et al. 2014](#); [Coghi et al. 2018](#)).

2. Results and discussion

Concerning their antiviral application, artemisinins have been extensively studied as potential remedies against herpes and hepatitis B and C viruses (Ho et al. 2014). As anticipated, artemisinins possess several pharmacological properties, and different mechanisms, such as anti-inflammatory action, reduction of nucleoprotein production or inhibition of viral RNA and proteins at post-entry step, may be involved in their antiviral effect, also in the case of SARS-CoV-2 (Cao et al. 2020; Kshirsagar and Rao 2021). The current study focuses on the investigation of the direct effect of artemisinin and dihydroartemisinin on S protein RBD. Computational techniques, including docking and molecular dynamics (MD) simulations, were adopted to study the structural details and the stability of the RBD-ligand complexes (Figure 1B). Moreover, the binding was tested *in vitro* by Bio-layer interferometry, a label-free technology for measuring biomolecular interactions.

Most of previous contributions investigated the docking of small molecules to RBD focusing on the interface with ACE2 (Rathod et al. 2020), but the presence of other sites also emerged (Alexpandi et al. 2020; Basu et al. 2020). Accordingly, binding

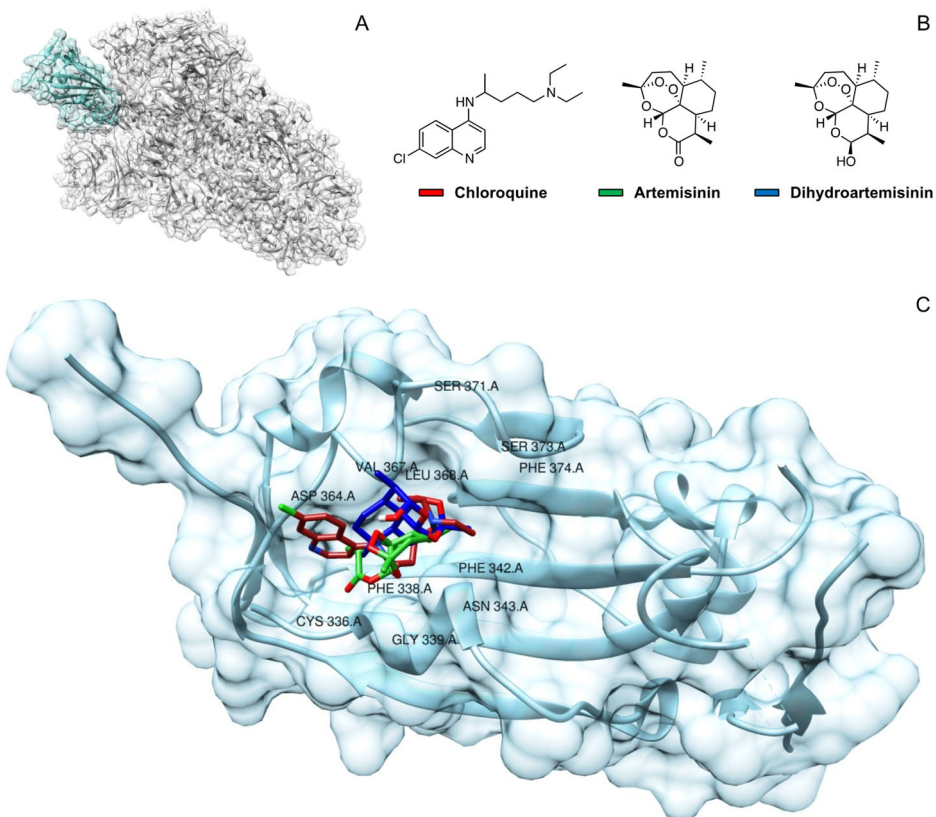


Figure 1. Spike protein trimer, with one of the RBDs rotated up and highlighted (PDB ID: 6VSB, A); chemical structures of the studied compounds (B); predicted interaction patterns with RBD of chloroquine (red, -5.6 kcal/mol), artemisinin (green, -7.0 kcal/mol) and dihydroartemisinin (blue, -6.5 kcal/mol, C).

surface analysis showed the presence of 3 putative ligand interaction sites on RBD (Figure S1 in the [Supplementary Material](#)). As previously observed in studies carried out on a structure obtained by homology modeling, docking highlighted artemisinin as the most promising ligand of the set (Figure 1C, see [Supplementary Material](#) for docking protocol) (Prashantha et al. 2021). The predicted binding models show that the compounds interact with the same region of RBD (Figure S2 in the [Supplementary Material](#)).

MD studies were enrolled to further investigate the stability of complexes (Doerr et al. 2016, Gianoncelli et al. 2020). In particular, 25 ns MD simulations on RBD-artemisinin, RBD-dihydroartemisinin and RBD-chloroquine docked models were carried out and root mean square deviation (RMSD) trajectory were compared. In all cases, the complexed protein backbones reached stabilization within less than 5 ns of simulation time: RMSD values (average \pm standard deviation) of $2.51 \pm 0.24 \text{ \AA}$, $2.15 \pm 0.18 \text{ \AA}$ and $2.33 \pm 0.14 \text{ \AA}$ were measured for backbones of RBD-artemisinin, RBD-dihydroartemisinin and RBD-chloroquine complexes, respectively. Greater difference was observed on the behavior of the ligands. In the RBD-chloroquine complex, the ligand reached stabilization after 7 ns and showed limited fluctuation throughout the remaining simulation time, in which the compound was retained within the binding site (average RMSD = $8.62 \pm 2.97 \text{ \AA}$). Similarly, dihydroartemisinin reached stabilization after 3 ns (average RMSD = $5.23 \pm 0.76 \text{ \AA}$). On the other hand, greater vibrations were observed for artemisinin, in particular in the 0-20 ns simulation timeframe, and the compound reached stabilization only in the final 5 ns of simulation (Figure S3 in the [Supplementary Material](#)).

The binding of chloroquine, artemisinin and dihydroartemisinin was then experimentally investigated using Bio-layer interferometry. This analysis relies on the immobilization of RBD to the biosensor surface with subsequent exposure to different ligand concentration, allowing the real-time measurement of association and dissociation phases (see [Supplementary Material](#) for experimental details). Chloroquine dose-dependently binds RBD ($K_D = 35.9 \mu\text{M}$, $R^2 = 0.9827$) more tightly than artemisinin ($K_D = 51.4 \mu\text{M}$, $R^2 = 0.6264$), while dihydroartemisinin showed weaker binding and worse correlation ($K_D = 66.5 \mu\text{M}$, $R^2 = 0.5210$). Kinetic parameters, such as association rate (k_{on}) and dissociation rate (k_{dis}) for these complexes are reported in the [Supplementary Material](#) (Figures S4–S6). Since ACE2 is the macromolecular interactor of RBD and small molecules interfering with such receptor could contrast viral infection (Bourgonje et al. 2020), binding of studied compounds was also measured towards ACE2. Although, no notable interaction was detected for chloroquine, artemisinin and dihydroartemisinin under these experimental conditions (Figures S7 and S8 in the [Supplementary Material](#)).

3. Conclusions

While docking studies suggested that artemisinin would have been the most promising binder for RBD, Bio-layer interferometry highlighted chloroquine as the best ligand of the set. Consistently, MD simulations confirmed that chloroquine forms a stable complex with RBD, as lower fluctuation values were measured for this assembly. The

results of this study support the hypothesis that “artemisinin” may act through a combination of mechanisms when exploiting their antiviral function, and suggest that RBD, rather than ACE2, could be one of the macromolecular targets for contrasting cellular invasion by SARS-CoV-2.

Disclosure statement

The authors declare no conflict of interest.

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