Exploring SARS-CoV-2 Delta variant spike protein receptor-binding domain (RBD) as a target for tanshinones and antimalarials

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ABSTRACT
The interaction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD) of spike protein with angiotensin-converting enzyme 2 (ACE2) mediates cell invasion. While this interaction mechanism is conserved, the RBD is affected by amino acid mutations in variants such as Delta and Omicron, resulting in enhanced transmissibility and altered ligand binding. Tanshinones are currently investigated as multi-target antiviral agents, but the studies were limited to the original SARS-CoV-2. This study aims at investigating the interaction of tanshinones with the Delta RBD. Chloroquine, methylene blue and pyronaridine, antimalarials previously identified as SARS-CoV-2 RBD binders, were studied for reference. Docking indicated the best scores for tanshinones, while bio-layer interferometry and molecular dynamics highlighted methylene blue as the best Delta RBD binder, although with decreased affinity with respect to the original strain.
1. Introduction

Coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Batah and Fabro 2021). While vaccines were made available in 2020, high hopes come from novel orally bioavailable antivirals approaching the market, such as molnupiravir and paxlovid (Wang and Yang 2021; Whitley 2022). Nevertheless, clinical trials showed lower-than-expected efficacy (Dyer 2021, Kozlov 2021), and the quest for promptly available compounds to combat infection by SARS-CoV-2 and its variants is still an urgent task. The interaction of a specific portion of SARS-CoV-2 spike protein (S), named receptor-binding domain (RBD, residues 331–524), with angiotensin-converting enzyme 2 (ACE2) mediates the host cell invasion (Mercurio et al. 2021). Thus, targeting the RBD with small molecules could prevent infection (Al Adem et al. 2020). Importantly, while this entry mechanism is conserved among coronaviruses (Li et al. 2003), RBD is affected by amino acid mutations in SARS-CoV-2 variants, and this results in altered viral transmissibility, pathogenicity, and immune escape (Tian et al. 2021). The Delta variant (B.1.617.2), classified as “variant of concern” by the WHO in May 2021, was reported to be more transmissible and become dominant (Tian et al. 2021). Similarly, Omicron (B.1.1.529) recently emerged as a novel and rapidly spreading “variant of concern” (Kumar et al. 2021). More specifically, the RBD of Delta variant bears L452R and T478K mutations, together with K417N in Delta+ (B.1.617.2.1) (Kannan et al. 2021).

While tanshinones were previously studied as SARS-CoV protease inhibitors (Park et al. 2012), their potential against SARS-CoV-2 was not fully explored yet. Cryptotanshinone, tanshinone I and tanshinone IIA are the main constituents of Tanshen (Salvia miltiorrhiza, 22 mg of tanshinones per gram of dry weight of hairy roots), used in Chinese medicine against cerebrovascular, cardiovascular and inflammatory diseases (Contreras et al. 2019; Ngo et al. 2021). Tanshinones were reported to possess several other biological activities, such as antioxidant, antibacterial and antimalarial activity (Sairafianpour et al. 2001; Zeng et al. 2016; Marrelli et al. 2021). Tanshinones represent another example of small molecules active as antimalarial drugs, a class that was early studied in a repurposing attempt to combat SARS-CoV-2 (Bojadzic et al. 2020; Krishna et al. 2021). We also previously screened a set of natural and synthetic antimalarials against the RBD of the original SARS-CoV-2 strain (Coghi et al. 2021; Ribaudo et al. 2021). With the current work, we aim at studying the interaction of tanshinones and of the most promising antimalarial drugs with the Delta RBD.

2. Results and discussion

Mutations in the Delta RBD lead to an overall structural change and a modification of the electrostatic surface of the protein. In fact, mutations introducing basic residues with positive charges characterize this variant (L452R and T478K), thus enhancing the affinity for ACE2 and increasing transmissibility and pathogenicity (Harvey et al. 2021; Tian et al. 2021). Moreover, such mutations could also alter the efficacy of ligands targeting the RBD. In this study, we investigate the inhibitory interactions of
cryptotanshinone, tanshinone I and tanshinone IIA (Figure 1) with Delta variant RBD by means of computational techniques and in vitro bio-layer interferometry studies. Despite having been identified as selective SARS-CoV cysteine protease inhibitors acting in the μM range in 2012 (Park et al. 2012), tanshinones did not receive full attention in the context of drug repurposing against SARS-CoV-2 until very recently. Tanshinone I was included in a virtual screening study on natural compounds against multiple SARS-CoV-2 targets (Hossain et al. 2021); meanwhile, cryptotanshinone, tanshinone I and the derivative tanshinone IIA sulfonate sodium (IC₅₀ = 1.65 ± 0.13 μM) were studied as a papain-like protease inhibitors (Xu et al. 2021; Zhao et al. 2021). Elebeedy et al. analyzed the interaction of tanshinone IIA with S protein in silico, and demonstrated that the compound inhibits viral absorption in vitro, suggesting an interference with RBD-ACE2 complex formation (Elebeedy et al. 2021). For comparison, the antimalarials chloroquine, methylene blue and pyronaridine (Figure 1), which were identified as good binders for the original SARS-CoV-2 strain RBD (Bojadzic et al. 2020; Coghi et al. 2021; Ribaudo et al. 2021), were included in the current study.

According to molecular docking, binding energies for the interaction with the Delta RBD calculated for tanshinones (cryptotanshinone −6.8 kcal/mol, tanshinone I −7.0 kcal/mol, tanshinone IIA −6.8 kcal/mol) overcome those predicted for synthetic antimalarials (chloroquine −4.9 kcal/mol, methylene blue −5.6 kcal/mol, pyronaridine −6.7 kcal/mol; a lower calculated binding energy value indicates a more efficient interaction). Moreover, differently from what observed in our previous studies on the original SARS-CoV-2 strain RBD (Coghi et al. 2021; Ribaudo et al. 2021), the compounds interact with different regions of the protein: tanshinone I, chloroquine and methylene blue form a first cluster, tanshinone IIA and pyronaridine colocalize in a second one, and cryptotanshinonine binds to a third site (Figure 1). With reference to the models predicted for the original RBD, chloroquine binds the same region (residues 338–374), but with a lower efficacy. The same holds true for methylene blue, while pyronaridine, that interacted through the same pocket on the former RBD, binds the Delta RBD in the 390–518 region (Figures S1–S6 in the Supplementary Material). Overall, lower affinity was predicted for these compounds against the Delta RBD. Thus, even if the mutated residues are not directly involved, the binding affinity of compounds is affected by structural changes of the Delta RBD.
The interactions of all compounds with the Delta RBD were also tested \textit{in vitro} using bio-layer interferometry, where the target protein is immobilized on a biosensor surface with subsequent exposure to different ligand concentrations (Figures S9–S14 in the \textit{Supplementary Material}). Methylene blue dose-dependently binds the Delta RBD ($K_D = 5.51 \times 10^{-5}$ M, $R^2 = 0.9479$) even if less efficiently than the protein of the original virus strain ($K_D = 2.26 \times 10^{-7}$ M, $R^2 = 0.9864$) (Coghi et al. 2021), while other compounds showed weaker binding and/or worse correlation. Among tanshinones, the tested compounds did not show good affinity \textit{in vitro}, with modest correlation values for tanshinone IIA. Interestingly, chloroquine and pyronaridine, previously reported as \textmu M SARS-CoV-2 RBD binders (Coghi et al. 2021; Ribaudo et al. 2021), do not interact with the Delta RBD. This decreased binding efficacy is in line with the docking predictions.

Based on the abovementioned findings and on the results previously reported in the literature (Elebeedy et al. 2021; Park et al. 2012), the predicted affinity of tanshinone IIA with other targets was further investigated \textit{in silico} to explore a potential selectivity profile. In particular, a blind docking experiment on the original SARS-CoV-2 strain RBD showed that the ligand interacts more efficiently with this target ($\approx 7.2$ kcal/mol) when compared to the Delta RBD and, as observed for other compounds from the current study, a different region of the protein is involved (Figure S7A in the \textit{Supplementary Material}). The interaction of tanshinone IIA with ACE2 was also investigated ($\approx 8.4$ kcal/mol), as well as the binding to main protease ($\approx 6.8$ kcal/mol, Figures S7A and S7B in the \textit{Supplementary Material}), which was hypothesized as a target for tanshinone IIA in a previous study (Elebeedy et al. 2021). Computed values fall within a rather narrow range, and are in line with previous findings from our group, since lower calculated binding energy values are generally computed towards ACE2 with respect to the RBD (Coghi et al. 2021).

Molecular dynamics (MD) was enrolled as a complementary tool to further investigate the ligand-RBD complexes formed by the most promising compounds (Doerr et al. 2016). More in detail, preliminary MD simulations were performed on the predicted models to assess the stability of the computed ligand-target complex in a limited timeframe (25 ns), and root mean square deviation (RMSD) trajectories were analyzed. While the complexed protein backbones reached stabilization within less than 5 ns of simulation time with RMSD values (average \pm standard deviation) of $1.62 \pm 0.24$ Å for RBD-methylene blue, the difference was observed on the behavior of the ligands (Figure S8 in the \textit{Supplementary Material}). In fact, tanshinone IIA was not retained within the binding site and expulsion occurred after 12 ns from the beginning of simulation (Figure S8 in the \textit{Supplementary Material}). On the other hand, in the RBD-methylene blue complex, the ligand reached stabilization and showed very limited fluctuations throughout the simulation time, and thus the compound was retained within the binding site.

3. Conclusions

Docking results indicated tanshinones as promising binders for RBD, while bio-layer interferometry studies showed that methylene blue interacted with the protein more
efficiently, suggesting that docking \textit{per se} cannot identify hits correctly in this context. Nevertheless, MD simulations confirmed the experimental observation. Methylene blue partially retains its RBD-interfering properties, while tanshinones may act weakly but synergically on viral replication on multiple targets and not exclusively on RBD. Computational and experimental results shed light on how mutations on the RBD dramatically impact ligands binding and their efficacy, promoting the identification and development of more effective small molecules retaining activity towards evolving targets and further exploration of these findings \textit{in vitro}.

\textbf{Disclosure statement}

The authors declare no conflict of interest.

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