Tetracycline as an inhibitor to the coronavirus SARS-CoV-2

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Abstract

The coronavirus SARS-CoV-2 remains an extant threat against public health on a global scale. Cell infection begins when the spike protein of SARS-CoV-2 binds with the cell receptor, angiotensin-converting enzyme 2 (ACE2). Here, we address the role of Tetracycline as an inhibitor for the receptor-binding domain (RBD) of the spike protein. Targeted molecular investigation show that Tetracycline binds more favorably to the RBD (-9.40 kcal/mol) compared to Chloroquine (-6.31 kcal/mol) or Doxycycline (-8.08 kcal/mol) and inhibits attachment to ACE2 to a greater degree (binding efficiency of 2.98 kcal/mol·nm² for Tetracycline-RBD, 5.59 kcal/mol·nm² for Chloroquine-RBD, 5.16 kcal/mol·nm² for Doxycycline-RBD). Stronger Tetracycline inhibition is verified with nonequilibrium PMF calculations, for which the Tetracycline-RBD complex exhibits the lowest free energy profile along the dissociation pathway from ACE2. Tetracycline appears to target viral residues that are usually involved in significant hydrogen bonding with ACE2; this inhibition of cellular infection complements the anti-inflammatory and cytokine suppressing capability of Tetracycline, and may further reduce the duration of ICU stays and mechanical ventilation induced by the coronavirus SARS-CoV-2.

I. Introduction

The extreme urgency for therapeutics against the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) drives the review of existing drugs for their ability to inhibit the function of this virus13,14. Tetracycline has been proposed as a strong candidate against SARS-CoV-2 due to its lipophilic nature, anti-inflammatory response, as well as its ability to chelate zinc species on matrix metalloproteinases (MMPs). Tetracycline class antibiotics have also been shown to be effective in reducing the duration of ventilatory support and ICU stay from acute respiratory distress syndrome,5 and Doxycycline has been suggested to be an important component in combination therapy for its anti-viral properties.5 Tetracycline as well as a broad band of related antibiotics have been approved by the FDA.4,10 In this work, we quantify the performance of Tetracycline in inhibiting the binding of the SARS-CoV-2 spike protein to ACE2. Tetracycline is found to bind more favorably to the receptor binding domain (RBD) of the spike protein compared to Doxycycline or Chloroquine, which was included in this study as a baseline. The Tetracycline-RBD complex also displays lower binding efficiency to the human cell receptor ACE2.

II. Methods

The SARS-CoV 2 RBD, ACE2, Tetracycline, and Chloroquine molecular structures were obtained from RCSB PDB (6M0J, 2UXO, 4V2O, 2XRL).14,15,16,17 Missing hydrogen atoms were appended, after which structural preparation and molecular docking with full ligand and protein backbone flexibility were carried out using the Rosetta suite.18,19,20 The resulting complexes were inspected manually, after which the binding affinities of the best-scoring complexes were gauged us-
ing MM/PBSA calculations after 100 ns equilibrium molecular dynamics simulations\textsuperscript{11,12,13}. The potentials of mean force (PMF)\textsuperscript{22} along the dissociation pathway of these RBD complexes from ACE2 were found in LAMMPS\textsuperscript{13} using steered molecular dynamics after parameterization with CHARMM\textsuperscript{23}.

Jarzynski’s equality was employed to calculate the free energy profile for each RBD complex from 10 statistically independent trajectories\textsuperscript{27}.

III. Results and Discussion

Tetracycline exhibits higher binding affinity to the RBD in both blind and site-specific docking (-9.40 kcal/mol) compared to Doxycycline (-8.08 kcal/mol) or Chloroquine (-6.63 vs 6.31 kcal/mol) as delineated in Table 1. The amino acid residues of the RBD involved in hydrogen bonding with the Tetracycline molecule are Tyr 449, Asn 501, Gly 496, and Tyr 505 (Fig. 1), which have been shown to be crucial for the SARS-CoV 2 RBD in binding to ACE2 for cellular access\textsuperscript{8}. These four residues comprise major hot spots that form persistent hydrogen bonds with ACE2. Meanwhile, the amino acids of RBD that interact with Chloroquine in the site-specific configuration are Lys 356, Arg 454, Arg 466 and Arg 355, of which none are involved in extended hydrogen bonding with ACE2.

Tetracyline appears to bind preferably to polar or slightly lipophilic RBD residues, which comprise the majority of amino acids that form persistent hydrogen bonds with ACE2\textsuperscript{8}. Other tetracycline derivatives as Doxycycline or Minocycline are known to be more lipophilic\textsuperscript{3,4,9} and may therefore prefer nonpolar residues\textsuperscript{10} that are often buried beneath the solvent accessible surface area of the spike protein. Indeed, the RBD residues that have highest binding affinity to Doxycycline are Tyr 449, Gly 447, Val 445, Gly 496, of which only two overlap with RBD amino acids that engage in extended hydrogen bonding with ACE2. On the other end of the spectrum, Chloroquine targets clusters of charged residues on the RBD that do not actively participate in hydrogen bonding with the cell receptor ACE2.

The binding efficiency\textsuperscript{13} (magnitude of binding energy normalized by contact interface area) of the SARS-CoV2 RBD-ACE2 complex was found to be 7.58 kcal/(mol-mm\textsuperscript{2}). In the presence of the

![Figure 1: Interaction map of amino acid residues of the SARS-CoV 2 receptor binding domain (RBD) that have the highest binding affinity with Tetracycline. The Tyr 449, Asn 501, Gly 496 and Tyr 505 residues have also been shown to form persistent hydrogen bonds in maintaining the RBD-ACE2 complex.](image)

| Inhibitor to RBD | $\Delta G_{\text{bind}}$ (kcal/mol) | $\Delta G_{\text{bind}}$ (kcal/mol) |
|------------------|-----------------------------------|-----------------------------------|
| This work        | N/A                               | Prior literature                  |
| Tetracycline     | -9.40                             | [5.3, 7.1]                        |
| Doxycycline      | -8.08                             | N/A                               |
| Chloroquine      | -6.31                             | N/A                               |

Table 1: The binding free energy of small-molecule inhibitors to the SARS-CoV2 receptor binding domain (RBD). Tetracycline binds preferably to the RBD.
Figure 2: The hydrogen bonding lifetimes of binding site residues of the inhibited RBD with ACE2 sustained in 100 ns of simulation time, normalized by hydrogen bonding lifetimes in the uninhibited RBD-ACE2 complex.

To verify this statement, steered molecular dynamics simulations were carried out to find the potential of mean force (PMF) along a singular dissociation pathway for the inhibited and uninhibited RBD-ACE2 complexes. Figure 3 shows that the PMF for unbinding of the Tetracycline-RBD complex from ACE2 was lowest of the three structures tested, which is in agreement with the binding efficiencies found from equilibrium simulations. This disruption of the RBD-ACE2 interface may therefore inhibit the signaling cascade initiated during binding of the viral spike protein.

Table 2: The binding efficiency (magnitude of binding energy normalized by contact interface area) of the spike protein RBD as well as the Tetracycline-RBD, Doxycycline-RBD and Chloroquine-RBD complexes to the human cell receptor ACE2. Binding efficiency is lowest for the Tetracycline-RBD complex, indicating that Tetracycline is a more effective inhibitor.

| Complex to ACE2           | Binding efficiency (kcal/(mol-nm^2)) |
|---------------------------|--------------------------------------|
| RBD                       | 7.58                                 |
| Chloroquine-RBD           | 5.59                                 |
| Doxycycline-RBD           | 5.16                                 |
| Tetracycline-RBD          | 2.98                                 |

Figure 3: The potential of mean force (PMF) as a function of the distance between the centers of masses of the spike protein RBD complexes and the cell receptor ACE2. The Tetracycline-RBD complex exhibits the lowest free energy profile along the dissociation pathway.
IV. Conclusion

The tetracycline class of antibiotics, including Tetracycline, Oxytetracycline, and Doxycycline may be helpful in the fight against the coronavirus SARS-CoV-2, due to its preferential association with the important residues in the viral receptor binding domain and the resulting strong inhibition of the RBD-ACE2 complex. Further experimental studies are recommended to validate how this reduction of cellular infection complements or enhances the anti-inflammatory and anti-viral properties of tetracyclines in their role as treatment for SARS-CoV-2.

Author contributions

T.Y.Z conceived and planned the research, as well as performed calculations. N.A.P. and T.Y.Z. performed analysis and wrote the manuscript.

Competing interests

The authors have no competing financial interests or other interests that might be perceived to influence the results and/or discussion reported in this paper.

References

[1] Omar, S.; Bouziane, I.; Bouslama, Z.; Djemel, A. In Silico Identification of Potent Inhibitors of COVID-19 Main Protease (Mpro) and Angiotensin Converting Enzyme 2 (ACE2) from Natural Products: Quercetin, Hispidulin, and Cirsimaritin Exhibited Better Potential Inhibition than Hydroxy-Chloroquine Against COVID-19 Main Protease Active Site and ACE2. 2020,

[2] Amin, M.; Abbas, G. Docking study of chloroquine and hydroxychloroquine interaction with RNA binding domain of nucleocapsid phospho-protein - an in silico insight into the comparative efficacy of repurposing antiviral drugs. J Biomol Struct Dyn 2020, 1–13.

[3] Sodhi, M.; Etminan, M. Therapeutic Potential for Tetracyclines in the Treatment of COVID-19. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2020, 40, 487–488.

[4] Yates, P. A.; Leone, A. M.; Reichel, E. A Proposed Randomized, Double Blind, Placebo Controlled Study Evaluating Doxycycline for the Prevention of COVID-19 Infection and Disease In Healthcare Workers with Ongoing High Risk Exposure to COVID-19. medRxiv 2020,

[5] J. D. Byrne, R. Shakur, J. Collins, S. L. Becker, C. C. Young, H. Boyce, and C. Traverso. Prophylaxis with tetracyclines in ards: Potential therapy for covid-19-induced ards? medRxiv, 2020.

[6] A. E. Malek, B. P. Granwehr, and D. P. Kontoyiannis. Doxycycline as a potential partner of covid-19 therapies. IDCases, 21:e00864, 2020.

[7] Andrei, S.; Droc, G.; Stefan, G. FDA approved antibacterial drugs: 2018-2019. Discoveries (Craiova, Romania) 2019, 7, e102–e102.

[8] Veeramachaneni, G. K.; Thunuguntla, V. B. S. C.; Bobbillapati, J.; Bondili, J. S. Structural and simulation analysis of hotspot residues interactions of SARS-CoV 2 with human ACE2 receptor. Journal of Biomolecular Structure and Dynamics 2020, 1–11.
[9] Du, Q.-S.; Li, D.-P.; He, W.-Z.; Chou, K.-C. Heuristic molecular lipophilicity potential (HMLP): Lipophilicity and hydrophilicity of amino acid side chains. *Journal of Computational Chemistry* 2006, 27, 685–692.

[10] Gautieri, A.; Beeg, M.; Gobbi, M.; Rigoldi, F.; Colombo, L.; Salmona, M. The Anti-Amyloidogenic Action of Doxycycline: A Molecular Dynamics Study on the Interaction with AB42. *Int J Mol Sci* 2019, 20.

[11] Nimgampalle, M.; Devanathan, V.; Saxena, A. Screening of Chloroquine, Hydroxychloroquine and its derivatives for their binding affinity to multiple SARS-CoV-2 protein drug targets. *Journal of biomolecular structure & dynamics* 2020, 1–13.

[12] Rohan Narkhede R., J. A. P.; Rameshwar Cheke S.; D., S. S. The Molecular Docking Study of Potential Drug Candidates Showing Anti-COVID-19 Activity by Exploring of Therapeutic Targets of SARS-CoV-2. *EJMO* 2020, 4, 185–195, doi: 10.14744/ejmo.2020.31503.

[13] Day, E. S.; Cote, S. M.; Whitty, A. Binding efficiency of protein-protein complexes. *Biochemistry* 2012, 51, 9124–9136.

[14] Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; Wang, X. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020, 581, 215–220.

[15] Alguel, Y.; Meng, C.; Terán, W.; Krell, T.; Ramos, J. L.; Gallegos, M.-T.; Zhang, X. Crystal structures of multidrug binding protein TtgR in complex with antibiotics and plant antimicrobials. *Journal of molecular biology* 2007, 369, 829–840.

[16] Huta, B. P.; Mehlenbacher, M. R.; Nie, Y.; Lai, X.; Zubieta, C.; Bou-Abdallah, F.; Doyle, R. P. The Lysosomal Protein Saposin B Binds Chloroquine. *ChemMedChem* 2016, 11, 277–282.

[17] Palm, G. J.; Buchholz, I.; Wertens, S.; Girbardt, B.; Berndt, L.; Delcea, M.; Hinrichs, W. Thermodynamics, cooperativity and stability of the tetracycline repressor (TetR) upon tetracycline binding. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 2020, 1868, 140404.

[18] Davis, I. W.; Baker, D. RosettaLigand docking with full ligand and receptor flexibility. *J Mol Biol* 2009, 385, 381–392.

[19] Lemmon, G.; Meiler, J. Rosetta Ligand docking with flexible XML protocols. *Methods Mol Biol* 2012, 819, 143–155.

[20] Meiler, J.; Baker, D. ROSETTALIGAND: protein-small molecule docking with full side-chain flexibility. *Proteins* 2006, 65, 538–548.

[21] Berendsen, H. J. C.; van der Spoel, D.; van Drunen, R. GROMACS: A message-passing parallel molecular dynamics implementation. *Computer Physics Communications* 1995, 91, 43–56.

[22] Lindahl, E.; Hess, B.; van der Spoel, D. GROMACS 3.0: a package for molecular simulation and trajectory analysis. *Molecular modeling annual* 2001, 7, 306–317.

[23] Kumari, R.; Kumar, R.; Lynn, A. g_mmpbsa—A GROMACS Tool for High-Throughput MM-PBSA Calculations. *Journal of Chemical Information and Modeling* 2014, 54, 1951–1962.

[24] Chen, P.-C.; Kuyucak, S. Accurate determination of the binding free energy for KcsA-charybdotoxin complex from the potential of mean force calculations with restraints. *Biophysical journal* 2011, 100, 2466–2474.

[25] Plimpton, S. Fast Parallel Algorithms for Short-Range Molecular Dynamics. *Journal of Computational Physics* 1995, 117, 1 – 19.

[26] Brooks, B. R. et al. CHARMM: the biomolecular simulation program. *J Comput Chem* 2009, 30, 1545–1614.

[27] Dellago, C.; Hummer, G. Computing Equilibrium Free Energies Using Non-Equilibrium Molecular Dynamics. *Entropy* 2013, 16, 41–61.