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MAJOR ARTICLE

In Silico Evaluation of Cyclophilin Inhibitors as Potential Treatment for SARS-CoV-2

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Background. The advent of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) provoked researchers to propose multiple antiviral strategies to improve patients' outcomes. Studies provide evidence that cyclosporine A (CsA) decreases SARS-CoV-2 replication in vitro and decreases mortality rates of coronavirus disease 2019 (COVID-19) patients. CsA binds cyclophilins, which isomerize prolines, affecting viral protein activity.

Methods. We investigated the proline composition from various coronavirus proteomes to identify proteins that may critically rely on cyclophilin's peptidyl-proline isomerase activity and found that the nucleocapsid (N) protein significantly depends on cyclophilin A (CyPA). We modeled CyPA and N protein interactions to demonstrate the N protein as a potential indirect therapeutic target of CsA, which we propose may impede coronavirus replication by obstructing nucleocapsid folding.

Results. Finally, we analyzed the literature and protein–protein interactions, finding evidence that, by inhibiting CyPA, CsA may impact coagulation proteins and hemostasis.

Conclusions. Despite CsA's promising antiviral characteristics, the interactions between cyclophilins and coagulation factors emphasize risk stratification for COVID patients with thrombosis dispositions.

Keywords. ADAMTS13; antiviral; coagulopathy; COVID-19; cyclophilin; cyclosporine A.

As of November 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–induced pandemic has infected 54 million people and claimed over 1 million lives worldwide (WHO). The virus's common infectious process begins with inhalation of SARS-CoV-2-containing aerosols or droplets that carry higher viral levels comparatively [1]. Aerosols, despite displaying lower capacity for virus, may pose a greater threat, as they travel farther distances to reach a novel host—resulting in viral binding with the spike protein to the angiotensin-converting enzyme II receptor in the respiratory epithelium and endocytosis of the virus [1, 2]. In severe cases of SARS-CoV-2 infections, the host immune response leads to a hyperinflammatory state characterized by systemic cytokine release, distinctly affecting the respiratory system and in severe cases evolving into acute respiratory distress syndrome (ARDS) [3].

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The use of cyclophilin (CyP) inhibitors as antiviral therapies began with the discovery that cyclosporine A (CsA) benefitted HIV patients during the 1980s [4]. CyPs, specifically cyclophilin A (CyPA), support HIV infection by (1) associating with the capsid protein, (2) interacting with novel virions, and (3) enabling viral migration into the nucleus by isomerizing the proline residues within the R protein [5]. As the HIV life, cycle depends on the roles of CyPA, applying CsA's CyPA inhibitory action reduced viral replication in humans [5]. Importantly, in vitro results have shown that SARS-CoV-2, SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS-CoV), among other coronaviruses, are effectively inhibited by CsA [6, 7], and clinical results show decreased mortality in coronavirus disease 2019 (COVID-19) patients who were administered CsA for severe cases [8].

CyPs are highly conserved chaperones in prokarya and eukarya taxa and serve a pivotal role in folding cellular proteins [9]. These enzymes isomerize proline residues between the cis and trans conformation via peptidylprolyl isomerase [10]. Prolines limit folding rates due to kinetic restrictions caused by alpha carbon's pyrrolidone ring functional group forming a secondary amine group (imine) [11]. CyPs solve these restraints by catalyzing the reaction rate of proline isomerization by a factor of 10^3–10^6 [12]. Due to the critical role of CyPs in protein folding and the potential of viral reliance on them, these enzymes are potential therapeutic targets. Drugs such as CsA

and its orthologs interact with CyPs and form the CyP-CsA complex, inhibiting PPIase (Figure 1) [13]. This decreases the folding rate of proteins containing prolines and prevents them from achieving their functional state [13].

CsA regulates immune responses by blocking the role of CyPA in the calcineurin/Nuclear factor of activated t-Cells (NFAT) pathway for T-cell activation [14]. CsA enters the T cell, binds CyPA with high affinity, and forms the CsA-CyPA complex [14]. This complex interacts with calcineurin, a Ca^{2+}-calmodulin-dependent serine-threonine-specific protein phosphatase, inhibiting its alpha subunit [14]. Consequently, calcineurin cannot activate T cells by dephosphorylating NFAT, which activates interleukin (IL)-2, IL-4, and CD40L transcription [14]. While this immunosuppressive activity of CsA could help prevent the complication of cytokine storm in COVID-19, it can also cause adverse coagulopathic effects such as CsA-induced thrombotic thrombocytopenic purpura or affect Na^{+}-Ca^{+} exchanger transporter activity [15].

Due to CsA's inhibition of diverse coronavirus replication, its known interaction with CyPA, and PPIase activity, we analyzed the proline composition of SARS-CoV-2 and related coronavirus proteomes. Our investigation highlights the nucleocapsid (N) protein of SARS-CoV-2 (the viral protein with the greatest proline density) and N proteins of other similar coronaviruses as the primary targets of CyPA. Our analysis of cis/trans prolines in the SARS-CoV-2 N protein and molecular docking experiments with CyPA point specifically to 2 prolines, P199 and P364, which may depend on the PPIase activity of CyPA to achieve the proper cis conformation. This points to a pivotal role of CyPA PPIase activity in N protein folding and viral propagation. We found that coagulation proteins interact relatively closely with both SARS/SARS-CoV-2 proteins and cyclophilins, implicating the latter in mechanisms of coagulation. We believe that this drug offers encouraging results with patient prognosis, but we contextualize our optimism with the potential risk of enhanced thrombotic events observed during CsA treatment for SARS-CoV-2. Nevertheless, our study offers promising insights regarding the use of CsA as a therapy for COVID-19.

METHODS

Coronavirus and Human Proteome Proline Analysis

We determined proline content in each protein from the SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-229E, HCoV-NL63, and human proteomes from reference sequences NC_045512.2, NC_004718.3, NC_019843.3, NC_002645.1, NC_005831.2, and CoCoPUTs, respectively [16, 17]. Proline density, defined as the number of prolines divided by the total number of amino acids, was measured for each virus and its proteins.

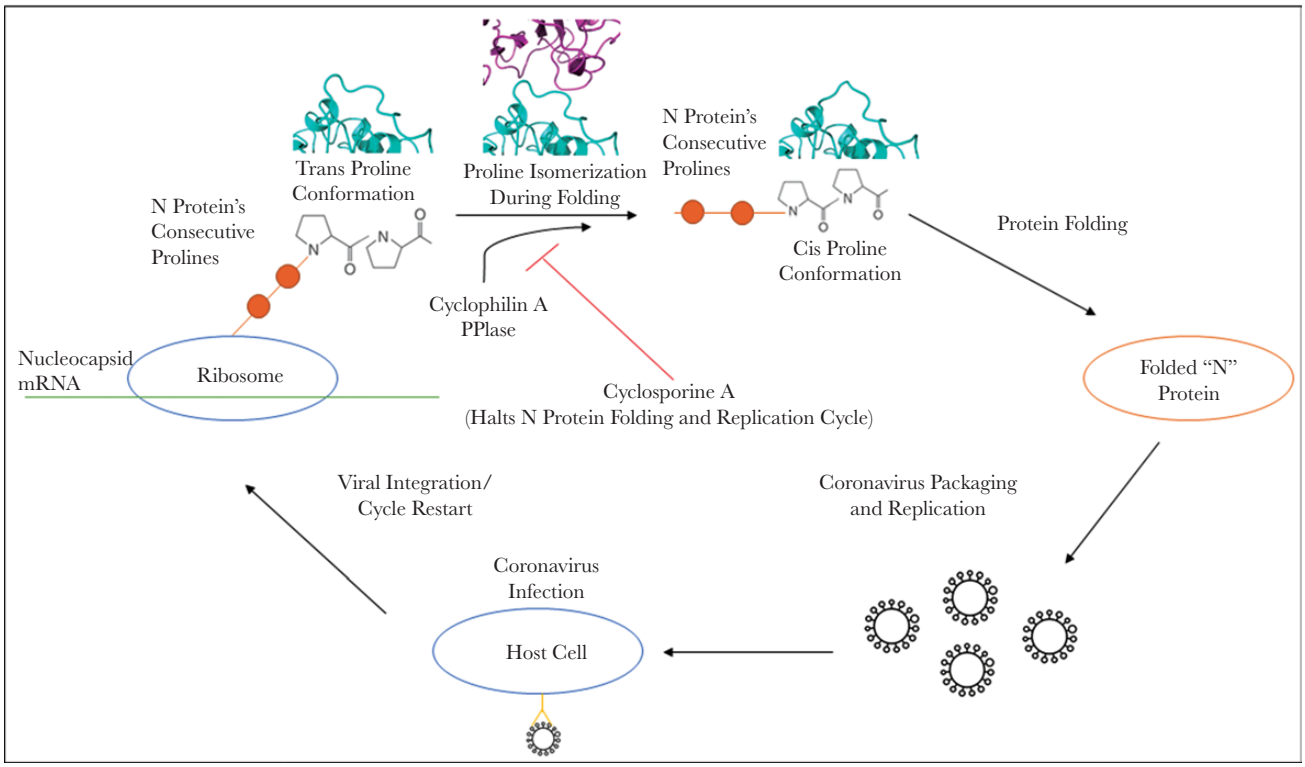

Figure 1. CsA PPIase inhibitory mechanism against coronavirus. The illustration above depicts our proposed mechanism behind CsA's inhibition of diverse coronaviruses. CsA blocks cyclophilin A's PPIase activity by forming the CsA–cyclophilin A complex, which we predict halts N protein folding to ultimately inhibit viral replication. Abbreviation: CsA, cyclosporine A.

SARS-CoV-2, MERS-CoV, SARS-CoV N Protein Analysis

Sequence similarity was measured by aligning each pair of sequences and computing the fraction of positions matching, excluding insertions and deletions, with the N protein's reference sequences for SARS-CoV, MERS-CoV, and SARS-CoV-2.

We computed the fraction of matching positions and log-likelihood based on the amino acid distribution at each position, derived from a multiple sequence alignment using 913 sequences constructed using BLAST; on SARS N, excluding any sequences containing SARS-CoV-2 in the name [18].

Cis/Trans Proline

We filtered protein structures from the Protein Data Bank (PDB) with PISCES to produce nonredundant structures generated from x-ray crystallography [19, 20], yielding 14 409 PDBs. Computing secondary structure and dihedral angles for this data set using DSSP and Bio3D (excluding those that produced errors) yielded 13 702 PDBs and 3 410 560 amino acid positions [21, 22]. Excluding nonprolines resulted in 154 390 proline positions. We analyzed DSSP secondary structure summaries for over/underrepresentation of cis prolines relative to trans prolines. Other computed characteristics, primarily dihedral angles and accessible surface area, were analyzed.

We analyzed the distribution of amino acids in the surrounding sequence. Specifically, we computed the probability of finding the surrounding sequence in cis/trans prolines as $\prod_{i=-5}^{5} p(aa_i)$, where $p(aa_i)$ represents the frequency of amino acid aa_i at position i around cis/trans) prolines.

We analyzed known SARS-CoV-2 variants [23]. If cis-trans isomerization is necessary for proper folding at a proline site, we would expect selection against variants at that site. We further compared the geographic distribution of prolines against other amino acids.

Docking SARS-CoV-2 N Protein and CyPA

We constructed structural models of CyPA and SARS-CoV-2 N protein with I-TASSER webserver [24]. We identified multiple potential binding sites by (1) aligning SARS-CoV-2 N sequence with HIV-1 gag sequence taken from Uniprot, (2) aligning SARS-CoV-2 N sequence with the HIV capsid protein sequence (5JFB), (3) using the binding site in SARS N protein, (4) using only the known CsA binding positions of CyPA, (5) using a predicted cis proline at site 199, and (6) using a predicted cis proline at position 364 [25–27]. Excluding option (4), we manually moved each prospective binding site into position with the predicted CsA binding site of CyPA in Pymol. We applied Rosetta local docking to each prospective binding site with 1000 trials [28].

Cyclophilins, Coagulation, and SARS-CoV-2 Proteins

Using BIOGRID, we constructed the network of protein–protein interactions for all available human, SARS, and SARS-CoV-2 proteins. We acquired a list of all coagulation-involved proteins by gene ontology and all cyclophilins and cyclophilin-like proteins in this network. We measured the distance between each coagulation-involved protein and the closest SARS or SARS-CoV-2 protein. We repeated this process, using instead a curated subset of coagulation-involved proteins measured in standard blood tests, all proteins, and cyclophilins. Finally, we compared the distances of proteins using a chi-square test.

RESULTS

SARS-CoV-2 Proline Analysis

We determined the SARS-CoV-2 N protein to have the greatest density of proline residues (Table 1). As CyPs assist in protein folding by catalyzing proline conformational changes between cis and trans (Figure 1), proteins with the greatest proline density and conserved prolines in the cis conformation will likely show the greatest reliance on the PPIase activity of CyPs. As the N protein has the highest proline density of SARS-CoV-2 proteins (0.0668) and a limited length of 419 amino acids, we hypothesize that it relies the most on PPIase activity to reach its native and functional state (Table 1). We analyzed the proline composition of HCoV 229E, HCoV NL63, SARS-CoV, and MERS-CoV. We found that the N protein displaying the highest density of proline residues (SARS-CoV was the only outlier, as ORF9b protein portrays the greatest proline density at 0.082) (Supplementary Table 1). We hypothesize that the coronavirus N protein's relative shortness would fail to tolerate nonisomerized prolines and yield more catastrophic effects on structure than a more tolerant, longer protein.

Table 1. SARS-CoV-2 Proline Bases Proteome Analysis

Gene	Protein	Total Prolines	Total # Amino Acids:	Ratio: Prolines/Total AA
ORF7b	ORF7b	0	43	0
ORF6	ORF6 protein	1	61	0.0164
M	Membrane glycoprotein	5	222	0.0225
ORF10	ORF10 protein	1	38	0.0263
E	Envelope protein	2	75	0.0267
ORF1ab	ORF1a polyprotein	164	4405	0.0372
ORF1ab	ORF1ab polyprotein	274	7096	0.0386
ORF3a	ORF3a protein	12	275	0.0436
S	Surface glycoprotein	58	1273	0.0456
ORF7a	ORF7a protein	6	121	0.0496
ORF8	ORF8 protein	7	121	0.0579
N	N phosphoprotein	28	419	0.0668

From top to bottom, SARS-CoV-2 proteins are ranked in ascending order with the ratio of proline residues to the total number of amino acids. The N protein supersedes the entire proteome with a proline density of 0.0668. ORF7b protein depicts the least proline density with 0 proline residues.

Abbreviations: N, nucleocapsid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS, MERS, and SARS-CoV-2 N Protein Analysis

We performed a pairwise percent identity matrix to continue analysis of the N protein. We confirmed closer similarity between SARS-CoV and SARS-CoV-2 than either SARS relative to MERS-CoV (Table 2). Specifically, 51.5% and 51.1% of nucleotide positions match between the SARS-CoV-2 and SARS sequences relative to MERS, respectively (Table 2).

The multiple sequence alignment between the N protein of MERS, SARS, and SARS-CoV-2 depicted a close similarity between SARS-CoV and SARS-CoV-2 at 89% and a more distant relationship between SARS-CoV-2 and MERS-CoV at only 45% matching (Table 3). The relatively close relationship between the N proteins led us to test docking of CyPA to the viral N proteins.

N Protein and CyPA Docking

We provide the minimum interface energy with each proposed N protein binding site with CyPA (Table 4). The docking sites identified by Zdock and Luo et al [26] display the largest binding energy. Further, we generated Robetta models of the SARS-CoV-2 N protein to verify the presence of cis prolines. The prolines identified in cis conformation in the I-TASSER N protein model were predicted in trans conformation in the Robetta model. However, the cis prolines' segments have no structural homologs and high predicted error in the models. Manually sculpting cis prolines into trans conformation resulted in small structural changes with root-mean-squared deviation of 0.785Å for P199 and 0.519Å for P364. However, sculpting P199 resulted in conformation changes, particularly at P207 and P309, which became cis.

Prediction of Cis vs Trans Prolines From Sequence

Our analysis demonstrated that cis prolines had significantly different distributions of the surrounding amino acid sequence. Specifically, nearly half of cis prolines have no data for the successive positions, meaning they either appear at the end of the sequence or are followed by a segment without structural characterization. In addition, cis prolines are significantly more likely to be in bends and hydrogen-bonded turns (P = 2.26e-135), while trans prolines are more evenly distributed among secondary structures (Supplementary Table 2). Cis prolines also have a significantly higher solvent-accessible surface area (P = 1.64e-105) (Supplementary Table 3). While dihedral angles and other physical characteristics differ significantly between cis and trans prolines, we cannot determine these without structural information.

We predict P199 to have a higher solvent-accessible surface area. Both cis prolines at 199 and 364 were expected to have a secondary coil structure. P364 has a surrounding sequence that looks more like a cis proline than P199 (Supplementary Table 4). However, when excluding the subsequent positions, P199 looks more like a cis proline than P364. We identified 2 SARS-CoV-2 N protein variants at P199 and none at P364 (Table 5).

Coagulation-Related Proteins Interaction With Cyclophilins and SARS-CoV-2

Coagulation-related proteins including von Willebrand Factor, platelet receptors, and coagulation factors show a shorter distance to SARS and SARS-CoV-2 proteins in the interaction network than proteins overall (P = .003) (Figure 2A). Likewise, coagulation-related proteins show a shorter distance to cyclophilins than human proteins altogether (P = 1.26e-88) (Figure 2B). This suggests that coagulation-related proteins

Table 2. Percent Identity Matrix of N proteins of SARS, MERS, and SARS-CoV-2

Levenshtein Distance Matrix of N Proteins of SARS-CoV, MERS, SARS-CoV-2			
	SARS-CoV-2	SARS	MERS
SARS-CoV-2		*0.875*	*0.511*
SARS	**0.905**		*0.515*
MERS	**0.460**	**0.449**	

Numerical values in the percent identity matrix display the percent of matching positions that are not insertions or deletions when pairs of sequences are aligned. These quantities imply a high similarity of SARS-CoV to SARS-CoV-2 N protein and a partial outlier relationship to MERS-CoV. Bold and italic data depict the distances for nucleotide sequences and amino acid sequences, respectively.

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 3. MSA Log Likelihood N for SARS, MERS, and SARS-CoV-2

	Amino Acids		Nucleotides	
	Fraction Matching (0–1)	MSA Log Likelihood	Fraction Matching (0–1)	MSA Log Likelihood
SARS N	1	0	1	0
MERS N	0.448	–3.079	0.510.448	–2.651
SARS-CoV-2 N	0.891	0.530	0.8701	0.687

Multiple sequence alignment performed with the N for coronaviruses SARS, MERS, and SARS-2 indicates a high similarity between SARS-CoV and SARS-CoV-2 (89% matching residues). MERS, however, partially mirrors SARS-CoV-2 with only 44% aligning residues. Conservation results indicate the potential for a SARS homologue.

Abbreviations: MERS, Middle East respiratory syndrome; N, nucleocapsid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 4. Docking Energies Between N Protein and Cyclophilin A

Dock site	Minimum Interface Energy
Alignment with entire HIV-1 gag	–20.337
Alignment with HIV-1 gag PDB	–23.022
Luo et al., 2004	–15.852
Zdock	–17.941
Cis proline 199	–24.741
Cis proline 364	–20.71

Minimum energy of amino acids at the interface between SARS-CoV-2 nucleocapsid protein and cyclophilin A predicted from Rosetta local docking.

Abbreviations: N, nucleocapsid; PDB, Protein Data Bank; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 5. Cis Proline Predicted Features

	P199	P364
RSA (NetsurfP2)	0.76	0.553
ASA (NetsurfP2)	107.785	78.509
q3 (NetsurfP2)	C	C
q8 (NetsurfP2)	C	C
Flank sequence cis prob/trans prob	0.043	0.271
Flank sequence cis prob/trans prob (previous only)	1.532	0.621

Comparison of predicted cis proline features computed using only sequence information.

more closely interact with cyclophilins and SARS/SARS-CoV-2 proteins than general human proteins.

While the curated coagulation-related proteins also show close relationships with cyclophilins and SARS/SARS-CoV-2 proteins, the result is not significant due to smaller sample size (Supplementary Table 5).

Thrombophilia Associated With CsA Therapy and COVID-19 Infection

We investigated the independent clinical effects of CsA treatment and SARS-CoV-2 on coagulation biomarkers and discovered stark similarities in potential risks for thrombosis. SARS-CoV-2 infection and application of CsA both separately increase platelet aggregation rates, plasma fibrinogen concentration, D-dimer concentration, thrombus formation, and von Willebrand Factor (VWF) concentration (Supplementary Table 6).

Transplant Patients Treated With CsA who Have COVID-19

Available studies have examined the clinical outcome and rate of SARS-CoV-2 in the context of graft recipients prescribed immunosuppressants. We pooled data to serve as an indirect measure assessing CsA risk and potential benefits for treating SARS-CoV-2 infections. In total, 57 of the 262 patients received CsA during SARS-CoV-2 infection. Collectively, the data depict no difference in SARS-CoV-2 incidence among transplant patients relative to the general population [29]. Seven of the studies reported (1) no increase in the risk of graft dysfunction, (2) no correlation with mortality and graft presence, and (3) a benefit with CsA treatment for SARS-CoV-2 infections (Supplementary Table 7).

DISCUSSION

Our interest in the mechanism behind CsA antiviral characteristics was sparked by studies demonstrating the inhibition of SARS-CoV-2 and previously known infectious human coronavirus replication in vitro [30, 31]. In addition to Guisado-Vasco et al., a longitudinal study of transplant patients receiving CsA with SARS-CoV-2 infections represented another study population to identify potential benefits and risks of CsA therapy [8].

Transplant recipients receive a combination of immunosuppressant therapies, including calcineurin inhibitors such as CsA and steroids, to prevent graft rejection [32]. This cohort may provide evidence favoring CsA treatment against SARS-CoV-2. Contrary to expectations of increased COVID-19 rates in graft patients chronically treated with immunosuppressants, kidney transplant patients show both similar and decreased levels of COVID-19 infection rates relative to the general population [29]. The decrease in infection rate may imply a preventative characteristic of CsA against viral infections [8]. Systematic review of graft patient outcomes suggests that maintaining a full CsA regimen decreases mechanical ventilation and death rates with SARS-CoV-2 infections (Supplementary Table 7).

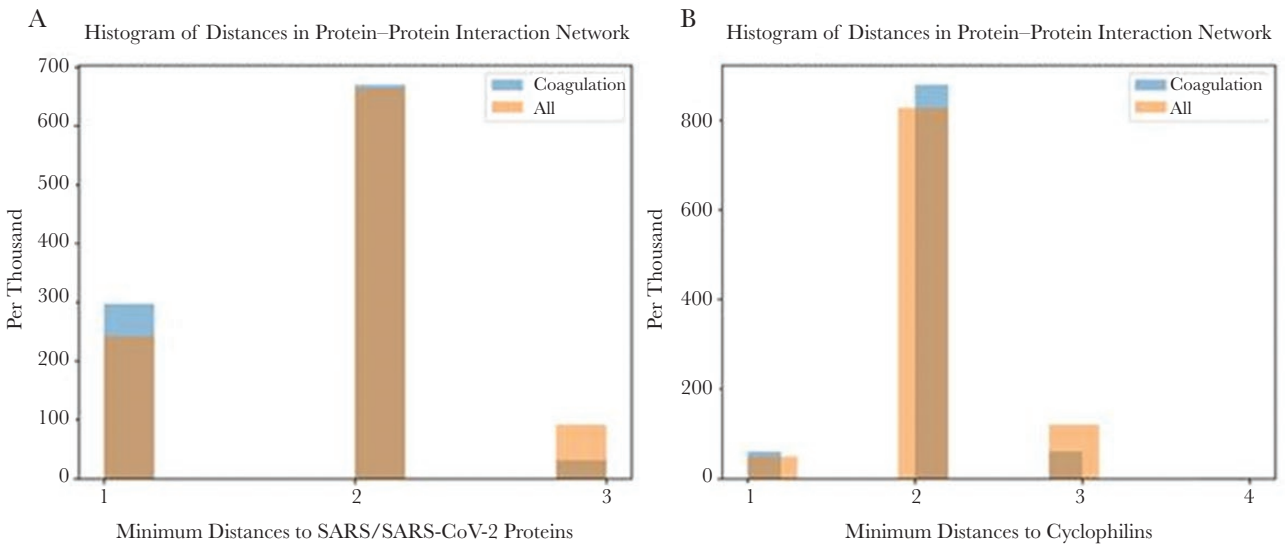

Figure 2. Histogram of shortest distances from proteins to cyclophilins and SARS/SARS-CoV-2 proteins. Coagulation proteins are significantly closer to cyclophilins (A) and SARS/SARS-CoV-2 proteins (B) than general human proteins in the protein–protein interaction network. This indicates a closer than average relationship between cyclophilins and SARS/SARS-CoV-2 proteins. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Unfortunately, transplant patients' medical complexity limits our assessment of CsA risks for treating SARS-CoV-2 independently [33].

We sought to identify similarities between coronaviruses by analyzing pairwise alignment of SARS-CoV-2, MERS-CoV, and SARS-CoV sequences. Alignments ranged from 50% to 79% similarity [34]. Given the variation in sequence homology, we expanded our literature search and identified lower N protein expression in CsA-treated groups [35]. Ma-Lauer et al. determined that CyPA associates with nascent N proteins, assists in folding, and subsequently enables viral replication, a process disrupted by CyP inhibitors [36].

As CyPs exhibit PPIase activity to isomerize proline residues, we analyzed proline distribution across the SARS-CoV-2 proteome and found that the N protein displays the greatest proline density (Table 1). Given the efficacy of CsA in other infectious coronaviruses, we investigated the relative proline density in HCoV 229E, HCoV NL63, SARS-CoV, and MERS-CoV. We identified a conserved N protein trend displaying the greatest proline density (SARS-CoV being the only outlier) (Supplementary Table 1). However, the difference between N and ORF9b is negligible when considering that ORF9 encodes the SARS-CoV N protein [37]. The evidence points to the N protein's reliance on the CyPA's PPIase activity, which is disrupted by CsA.

We modeled the N protein and noted that 2 "cis" proline residues (P199 and P364) in the folded protein surpassed all SARS-CoV-2 proteins. We modeled the protein with both prolines in the trans conformation to identify potential differences in structural importance. Relative to P364, altering P199 conformation demonstrated fewer disruptions to protein folding (Table 5). Such differences may stem from differential roles in protein solvation, as solvent accessibility decreases when cis prolines revert to trans conformations (Supplementary Table 3). Two known mutations occur at P199, but P364 is completely conserved (Table 5). This implies structural dependency on a "cis" P364. As Kern et al. demonstrated, CyP isomerases push the proline conformation's equilibrium in favor of the cis isomer [38]. Proline residues partake in various secondary structures, and motifs exhibit differential preferences for cis and trans conformations (Supplementary Tables 2 and 3). Therefore, inhibiting CyP activity with CsA will likely impede the N protein's proper folding due to its conserved cis proline.

We studied the interaction of CyPA with the N protein (Table 4). If the folding process cannot follow standard kinetics, nascent protein folding may terminate in a local energy minima and fail to reach the functional native state [39]. Improperly folded N proteins would fail to enclose viral RNA, transport complete virions, and infect surrounding tissue. Therefore, one would expect a decrease in viral replication and infection severity. Studies have observed these results from CsA treatment in vitro and in vivo [8, 30, 40]. Published data [40] appear to solidify our CyP-based mechanism as treatment of SARS-CoV-2-infected cells with nonimmunosuppressant CsA derivatives decreases viral replication [35].

To better characterize why CsA displays similar inhibitory responses when administered to diverse coronaviruses, we aligned the N protein reference sequences and analyzed their proline composition to quantify their similarity (Table 2; Supplementary Table 1). SARS-CoV and SARS-CoV-2 display 90% similarity, suggesting the conservation of CyPA docking and the misfolding of the N protein when administering CsA (Table 4).

Age appears to be a significant risk factor in SARS-CoV-2 infections [41], and it is known that CyP expression positively correlates with age [42]. We hypothesize that CyPA plays an integral role in the assembly of SARS-CoV-2 virions by assisting in N protein folding (Table 4). It is reasonable to assume that the increase in CyPA with age may proximately increase N protein formation and ultimately increase SARS-CoV-2 virulence in older hosts.

An additional significant drug interaction is the known association between CsA and VWF cleaving protease (ADAMTS13). ADAMTS13 is secreted by stellate hepatic cells into plasma to regulate the concentration of thrombogenic high–molecular weight VWF [43]. Administration of CsA decreases ADAMTS13 antigen and activity levels by associating with CyPB and hindering proper folding of ADAMTS13 [13]. Therefore, lowering ADAMTS13 levels in SARS-CoV-2 patients may potentially lead to thromboembolic complications as part of CsA therapy (Supplementary Table 6). The interactions between coagulation-related proteins, cyclophilins, and SARS-CoV-2 proteins should be considered (Figure 2), as these coagulation factors may be adversely affected by CsA.

It is imperative to account for atypical thrombosis and other hemostatic disruptions in determining the safety and efficacy of using CsA as a standard of care for COVID-19 patients. It will be interesting to examine the outcomes of CsA clinical trials for drug benefits while closely tracking the coagulation biomarkers previously mentioned. This study helps explain CsA's mechanism of action on SARS-CoV-2 and provides context for future studies into this promising therapy.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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