Identifying COVID-19 Drug-Sites Susceptible To Clinically Safe Zn-ejector Drugs Using Evolutionary/Physical Principles

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ABSTRACT. The Covid-19 outbreak requires prompt response, but developing Covid-19-specific antivirals/vaccine takes time. Near-term alternatives are needed. Based on evolutionary and physical principles of the key factors controlling the reactivity of Zn-bound Cys, we have identified putative labile Zn-sites in Covid-19 that can be targeted by Zn-ejector drugs, leading to Zn^{2+} release and viral structure/function disruption. We propose assessing the efficacy of FDA-approved Zn-ejector drugs such as disulfiram combined with interferon to treat Covid-19 infected patients.

An outbreak of respiratory sickness caused by a novel coronavirus (Covid-19) has become a serious public threat and disrupted many lives. Covid-19 has resulted in >73,500 confirmed cases in 29 countries and >1,800 deaths at the time of writing. These numbers are escalating, but no proven curative treatment for Covid-19 is available. However, the marketed drug pair (lopinavir-ritonavir) used to treat HIV infections showed anti-CoV activity in non-randomized trials of SARS patients,[1] and is being assessed for efficacy and safety in Covid-19 infected patients.[2, 3] This indicates the timeliness of repurposing FDA-approved drugs to treat Covid-19-infected patients since developing clinically useful Covid-19-specific therapeutics takes time. Immediate strategies that could save lives are desperately needed. Here, we identify putative druggable Zn-sites in three Covid-19 proteins and propose that Zn-ejector drugs such as disulfiram (an anti-alcoholism drug) can be used to disrupt Covid-19 protein structure/function (Figure 1).

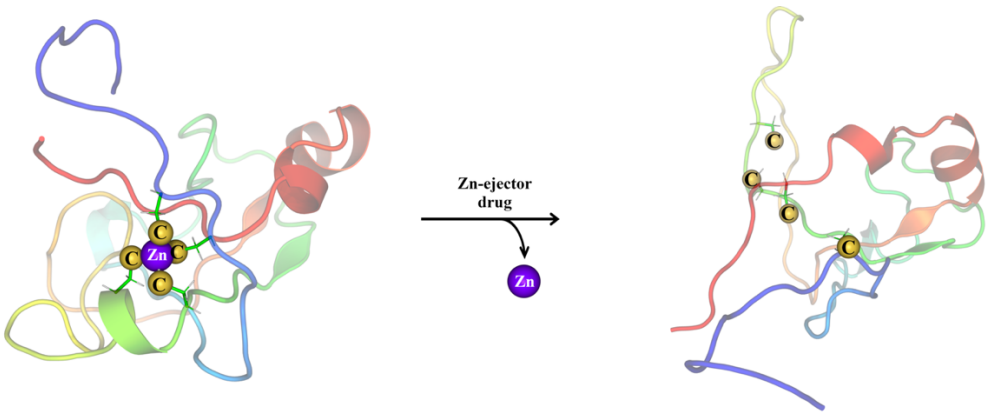

Figure 1. Schematic diagram to illustrate Zn^{2+} release by Zn-ejector drugs such as disulfiram.

Zn^{2+} is needed for the replication of many viruses and has been shown to play a crucial role in viral infections.[4] It is an essential cofactor in Zn-finger proteins by inducing protein folding and stabilizing the local structure.[5, 6] Certain Zn-finger cores can serve as therapeutic drug targets for viral infection.[7-10] These contain *labile* Zn-sites where the Zn-bound thiolates can react with Zn-

ejectors, leading to loss of viral protein structure/function (Figure 1). Our previous studies showed that such reactive Zn-bound cysteines are generally found in Zn-Cys_4 or Zn-Cys_3His sites lacking hydrogen bonds to any Zn-bound Cys(S^-), which would reduce the thiolate's negative charge and thus reactivity (Supplementary Figure S1).[9, 11] We then used these guidelines to identify new drug targets; i.e., proteins containing reactive cysteines bound to an essential structural Zn^{2+}.[10, 12]

We proposed that Zn-ejecting agents, FDA-approved or in clinical trials (Supplementary Table S1), can be used to target the predicted labile Zn-sites: We showed that disulfiram, a cysteine modifier,[13] could eject Zn^{2+} from the predicted labile Zn-Cys_4 site in the hepatitis C virus (HCV) NS5A protein, inhibiting viral replication, and that inhibition was enhanced when disulfiram was combined with interferon-α.[10] Subsequently, disulfiram was found to eject Zn^{2+} and inhibit replication in other viruses, notably MERS-CoV and SARS-CoV PLpro papain-like proteases (PLpro).[14] Based on these findings, our aim is to determine if the Covid-19 virus contains druggable Zn-sites that can be targeted by clinically used Zn-ejectors.

To identify putative Covid-19 druggable Zn-sites, we analyzed the virus genome (GenBank: MN908947.3) and focused on the replicase polyprotein pp1ab because its cleavage products, nonstructural protein (nsp) 7 to nsp16, serve to direct coronavirus RNA synthesis and processing.[15] Since viruses belonging to the order Nidovirales such as coronaviruses share conserved core replicase domains; e.g., RNA-synthesizing enzymes,[15] we searched the pp1ab sequence for conserved domains using the Conserved Domain Database[16] and found 18 such domains (Supplementary Table S2). Next, we searched the Protein Data Bank (PDB)[17] for < 3Å structures from other coronaviruses sharing similar function and high sequence identity to each conserved domain found in the Covid-19 polyprotein using BLASTp[18] (Supplementary Scheme S1). We then

checked each structure for Zn-(Cys_4/Cys_3His) sites lacking hydrogen bonds to the Zn-bound Cys(S^-).

Putative labile Zn-sites with no hydrogen bonds to all Zn-bound thiolates were found in SARS-CoV structures of PLpro cysteine protease (4m0w_A, 3e9s_A, 5tl7_B) and nsp10 Zn-finger protein (2xyq_B, 2fyg_A, 5c8u_A, 5nfy_O, 5nfy_P, 2ga6_F, 2ga6_R,5c8t_C). Such a labile Zn-site was also found in the recent SARS-CoV nsp13 helicase structure (6jyt_B). To obtain the corresponding Covid-19 nsp13 sequence, we aligned the SARS-Cov helicase and the Covid-19 pp1ab sequences using BLASTp[18] and obtained excellent alignment (Supplementary Table S3).

Table 1. Predicted Covid-19 Drug Target Proteins and their Templates for Building Models

nCoV domain[a]	Template[b]	Template protein	Zn-ligands in template	Known Zn-ejectors
PLpro	4m0w_A 1.4 Å (83%)	SARS-CoV PLpro	C190, C193, C225, C227	Disulfiram
nsp10	2xyq_B 2.0 Å (98%)	SARS-CoV nsp10 in complex with nsp16	C117, C120, C128, C130	None
nsp13 or helicase	6jyt_B 2.8 Å (99%)	SARS-CoV helicase	C50, C55, C72, H75	None

[a]Conserved domain found by Conserved Domain Database.[16] [b]PDB entry, chain ID and resolution of protein with highest % sequence identity with nCoV target protein in parentheses.[14]

Covid-19 nsp10/nsp13 and PLpro 3d-structures derived from the SARS-CoV structures in Table 1 using the SCRWL4[19] and MODELLER[20] program, respectively (Supplementary Figure S2) confirm the absence of hydrogen bonds to the Zn-bound thiolates. Furthermore, a 3d-structure of the Covid-19 PLpro with the catalytic cysteine covalently modified by disulfiram obtained using QM/MM energy minimization shows that the active site can accommodate a covalent adduct with the catalytic cysteine (Supplementary Figure S3).

To our knowledge, we are the first to combine knowledge of conserved coronavirus domains and the key factors controlling Zn-bound cysteine reactivity[11] to identify new drug sites in Covid-19. The labile Zn-sites discovered in Covid-19 PLpro, nsp10 and nsp13 are attractive drug targets, as they play vital structural/catalytic roles: The Zn-binding ability of PLpro, residing in nsp3, is crucial for structural integrity and protease activity.[21] The labile Zn-site in nsp10 transcriptional factor plays a critical structural role.[22] The Zn-binding domain of nsp13 helicase, which catalyzes dsRNA/dsDNA unwinding, is crucial for helicase activity.[23] We propose to disrupt these drug target protein structures/functions using Zn-ejector drugs such as disulfiram. Previous experimental studies support our proposal:[14] Disulfiram was found to inhibit SARS-CoV PLpro by ejecting Zn^{2+} thus destabilizing the protein and by modifying its catalytic cysteine.[14] Since the Covid-19 and SARS-CoV PLpro domains share high (83%) sequence identity and structural similarity, disulfiram may likewise inhibit Covid-19 PLpro by targeting conserved Zn-bound and catalytic cysteines (Figures 1 and 2).

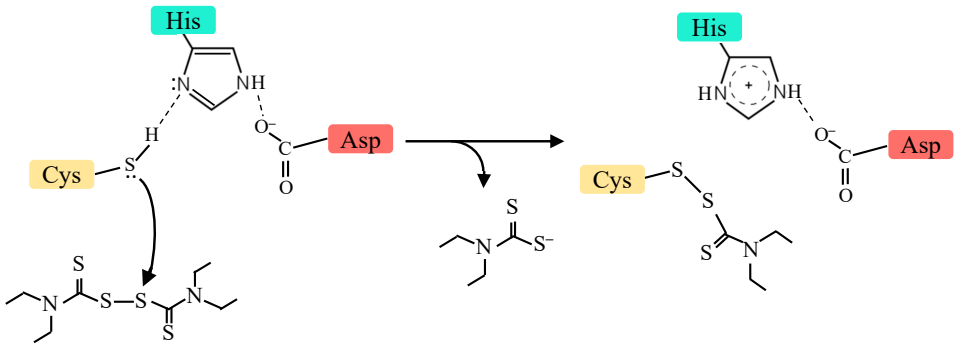

Figure 2. Schematic diagram to illustrate possible mechanism of attack by disulfiram in the cysteine protease active site

Disulfiram has been used since 1951 with a recommended daily dose of ≤500 mg. It is safe[24] and cheap (<US$2 per pill in Taiwan). In combination with interferon, disulfiram may

synergistically inhibit Covid-19 replication, as found for HCV.[10] Disulfiram may serve as a multi-target drug acting at various virus life cycle stages: It can inhibit Covid-19 replication by targeting Zn-bound cysteines in PLpro, nsp10 and nsp13, and catalytic cysteines in cysteine proteases (PLpro and 3CLpro) that catalyze polyprotein cleavage.[25] By targeting vital cysteines in multiple conserved domains, disulfiram might exhibit a higher barrier to Covid-19 resistance than some antiviral agents, as mutations of Zn-binding/catalytic cysteines would render the virus nonviable.[10]

In summary, this study provides a novel strategy combining conserved protein domains with the key factors controlling Zn-bound Cys reactivity to discover previously unknown druggable Zn-sites in Covid-19 PLpro, nsp10, and nsp13 domains. It presents an avenue for treating Covid-19-infected patients using clinically safe Zn-ejecting drugs to attack conserved catalytic and/or Zn-bound cysteines in multiple targets; thus, assessing their efficacy combined with interferon in clinical settings would be of great interest. Our strategy based on evolutionary and physical principles is general and can be used to identify druggable Zn-sites in conserved domains of other viruses. Importantly, it offers a possible strategy to tackle future outbreaks of pandemic viruses: FDA-approved drugs for a certain *conserved* domain may be repurposed to target the same *conserved* domain found in a new infectious virus. Furthermore, by targeting conserved domains with druggable Zn-sites, drugs may be used to treat several types of viruses.

ASSOCIATED CONTENT

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interests.

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ABBREVIATIONS

Covid-19, 2019 novel coronavirus; nsp, nonstructural protein; PDB, Protein Data Bank; PLpro, papain-like protease

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