Current Evidence Supporting the Use of Orally Administered Zinc in the Treatment of COVID-19

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Abstract:

This review presents current evidence supporting the following hypothesis:

> COVID-19 severity can be reduced with the administration of zinc in an orally and gastrointestinal absorbable form.

This supporting evidence (scientific premise) includes a variety of prior published work along with relevant data present in public scientific repositories that support the mechanistic idea that zinc concentrations in the oral mucosa, gastrointestinal tract, and possibly other parts of the human body can be elevated to the level that is inhibitory on the RNA-dependent RNA polymerase replication and transcription complex (RDRP-RTC) of SARS-CoV-2. There are several implications for this hypothesis. First, zinc represent a highly available nutrient that can be administered in the possibly therapeutic dosage range of 100 mg to 200 mg per day for short periods of time with no appreciable toxic effects. However, many zinc lozenges currently available on the market are not formulated to maximize oral absorption of zinc. But zinc can be quickly obtained, oral treatments produced, and then added to treatment protocols, even in developing nations. Second, oral zinc treatment may be synergistic with other drugs being actively studied and used in the treatment of COVID-19. Specifically, chloroquine is a known zinc ionophore and a possible mechanism of action for this drug and its similar derivatives is to increase zinc concentrations to a level that is inhibitory of SARS-CoV-2's RDRP-RTC, particularly in the lung epithelium. Moreover, dietary zinc and zinc depleting drugs could be confounding factors in current chloroquine and hydroxychloroquine clinical studies that are currently underway.

1. Introduction

COVID-19, which is caused by an infection of severe acute respiratory syndrome coronavirus 2 of the genus Betacoronavirus (SARS-CoV-2; previously called 2019-nCoV), is an imminent world health threat with huge economic repercussions. Given the acute crisis that is unfolding, effective and low-risk treatments are needed TODAY in order to reduce the impact of this global pandemic. Towards this end, the following hypothesis is presented:

> COVID-19 severity can be reduced with the administration of zinc in an orally and gastrointestinal absorbable form.

The foundation for this hypothesis is a 2010 paper by te Velthuis et al. which demonstrated the inhibition of the RNA-dependent RNA polymerase replication and transcription complex (RDRP-RTC) of SARS-CoV (severe acute respiratory syndrome coronavirus of the genus Betacoronavirus and causative agent of SARS) in Vero-E6 cell culture using 2 μM zinc and 2 μM pyrithione, a zinc ionophore (1). This paper and its possible implications have been bouncing around the internet since early February 2020 and has been touted in recent publications (2, 3). However, this author has not seen a prior detailed scientific premise presented to support a therapeutic use of zinc for COVID-19. Therefore, the goal of this review is to present a complete scientific premise for the proposed hypothesis and to discuss the possible implications for this hypothesis.

SARS-CoV and SARS-CoV-2 are both human coronaviruses and are each other's closest human viral strain with ~79% genomic sequence identity (4). There is some evidence that both viruses descended from bat coronaviruses, but the direct zoonotic ancestor for either virus has not been identified (5-10). SARS-CoV-2 is closely related (~88% genomic sequence identity) to three SARS-like bat coronaviruses: bat-SL-CoVZC45, bat-SL-CoVZXC21, and RaTG13 (7, 10).

2. Results and Discussion

There are five major groups of evidence that provide the scientific premise supporting this hypothesis:

1. Micromolar levels of intracellular zinc inhibit the RDRP-RTC of SARS-CoV.
2. Zinc is absorbed by human oral mucosa cells.
3. Zinc lozenges have demonstrated efficacy for treating common colds, which includes those caused by coronaviruses.
4. The human oral mucosa and gastrointestinal tract highly expresses the ACE2 receptor, which is targeted by SARS-CoV-2 for entry into cells with help from TMPRSS2.
5. A recent retrospective observational study demonstrates zinc's efficacy in treating COVID-19.

1. Micromolar levels of intracellular zinc inhibit the RDRP-RTC of SARS-CoV.

As mentioned previously, te Velthuis et al., 2010 demonstrated the inhibition of RNA synthesis by the RDRP-RTC in SARS-CoV infected Vero-E6 cells with 2 μM zinc (from zinc acetate) and 2 μM pyrithione, a zinc-ionophore. This paper also demonstrated similar inhibition of RDRP-RTC of the equine arteritis virus (EAV), which is an arterivirus. Furthermore, an in vitro enzyme activity assay demonstrated inhibition of RNA synthesis of both viruses' RDRP-RTCs with just zinc along with the removal of inhibition with the addition of MgEDTA, a zinc chelator (1).

Both arteriviruses and coronaviruses are members of the nidovirus taxon of viruses with animal and human hosts. An NCBI BLAST (11) search using the sequence of SARS-CoV-2's RDRP provided the following selected matches:

- QHR63299.1 - orf1ab polyprotein [Bat coronavirus RaTG13] at 100% coverage with 98.55% sequence identity.
- ACZ71930.1 - orf1ab polyprotein [SARS coronavirus wtic-MB] at 100% coverage with 86.09% sequence identity.
- NP_127506.1 - replicase ORF1ab polyprotein [Equine arteritis virus] at 10% coverage with 25.00% sequence identity (14% coverage with 24.86% identity to ACZ71930.1)

The closest match in the search is to the RDRP of bat coronavirus RaTG13 with 98.55% sequence identity. The SARS-CoV RDRP has 86.09% identity with the SARS-CoV-2 RDRP as opposed to 24.86% identity (and a much lower coverage) with the EAV RDRP. The low sequence identity (24.86%) between RDRPs of EAV and SARS-CoV, both of which have similar zinc inhibitory effects, support the idea that zinc will have a similar inhibitory effect on the SARS-CoV-2 RDRP-RTC, which has 86.09% sequence identity with the SARS-CoV RDRP. Furthermore, high structural similarity is demonstrated between RDRP (nsp12 protein) and RecA-like helicase (nsp13 protein) components of the SARS-CoV and SARS-CoV-2 RDRP-RTCs via structural modeling (12).

Which part of the RDRP-RTC is binding zinc for a replicative inhibitory effect is not known. However, the RecA-like helicases of SARS-CoV (nsp13 protein) and EAV (nsp10 protein) both contain a zinc binding domain (13-16). The SARS-CoV RecA-like helicase with this zinc binding domain has been structurally determined with bound zinc ions (16) and used to model the SARS-CoV-2 RecA-like helicase (nsp13 protein) bound to three zinc ions (12). Furthermore, site-directed mutagenesis of the zinc binding domain of EAV's RecA-like helicase demonstrates defective RNA synthesis and genomic replication. Specifically, mutation of nine of the ten completely conserved cysteines and histidines in the zinc-binding domain abolished viral replication (17). This abolishment of viral replication includes simple swapping of conserved cysteines for histidines and visa versa (17), which implies that specific zinc binding

conformations are required in this zinc binding cluster to enable viral replication. Therefore, one hypothetical structural mechanism is that high zinc concentration alters these zinc binding conformations, diminishing the ability of this protein to facilitate viral replication.

Another possible mechanism is the zinc-induced alteration of a host protein function required for viral replication. There are over 3000 putative human zinc binding proteins (18) and zinc is a well-established second messenger involved in many signaling processes (19). For example, high zinc concentration inactivates p38 mitogen-activated protein kinase (p38 MAPK) activity (20), which is required for viral replication of coronavirus HCoV-229E (21). In this example, a known zinc ionophore, chloroquine, inhibited p38 MAPK activity and viral replication in L132 human fetal lung cells (21). Moreover, many detected host proteins that interact with RDRP (nsp12 protein) and RecA-like helicase (nsp13 protein) of SARS-CoV-2 (22) are zinc binding proteins. In particular, SLU7 is a pre-mRNA splicing factor that was pulled down in an affinity-purification mass spectrometry assay with SARS-CoV-2's nsp12 protein as bate (22). SLU7 has a zinc knuckle motif that will localize most of the protein to the nucleus (23), except under certain cellular stress conditions (24). A weak zinc knuckle mutant of SLU7 mostly localizes to the cytoplasm, except under high zinc concentration (23). As another hypothetical mechanism, high zinc concentration could force SLU7 nuclear localization to a level that is disruptive to viral replication. Also, if zinc is altering the function of an essential host protein in viral replication, then viral mutation should not easily confer resistance to a zinc-based antiviral treatment.

2. Zinc is absorbed by human oral mucosa cells.

Kapadia et al., 2018 demonstrated the absorption of zinc by buccal mucosa cells from orthodontic appliances (25). This is an important direct observation supporting the efficacy of

oral administration of zinc via long-term zinc exposure, which is practical with zinc lozenges but not zinc supplementation in a pill form. While the direct mechanism of transport into oral lining mucosa has not been teased out, it is likely due to a zinc transporter expressed by the oral mucosa. One possible transporter is ZIP4, since it is the main transporter involved in zinc absorption in the gastrointestinal tract (26, 27). Moreover, ZIP4 knockout mice die unless fed a high-zinc diet, demonstrating that high zinc dosage can overcome zinc transporter deficiencies (28). Likewise, human acrodematitis enteropathica, caused by autosomal recessive mutations of human ZIP4 (*SLC39A4* gene, i.e. solute carrier family 39 member 4) which leads to zinc deficiency (29, 30), was successfully treated with oral zinc supplementation (3mg zinc per kg per day) in a recent pediatric case study (31). The implications from this case study and the mice ZIP4 knockout studies is that zinc can be absorbed from oral administration, without requiring the presence of a zinc transporter in the oral lining mucosa. Again, oral lining mucosa absorption of zinc was directly demonstrated by Kapadia et al., 2018 (25).

3. Zinc lozenges have demonstrated efficacy for treating common colds, which includes those caused by coronaviruses.

Zinc lozenges as a treatment to reduce the severity of symptoms for the common cold have been studied for almost 40 years since the first major double blind study published in 1984 by Eby et al. (32). Subsequent studies followed with a range of mixed results, causing a general disillusionment in the use of oral zinc lozenges. Finally, a rigorous meta-analysis of zinc lozenge efficacy was published in 2017 by Harri Hemilä that clearly demonstrated the efficacy in zinc lozenges to reduce the duration of common cold symptoms (33). This meta-analysis identified the addition of additives that reduced oral and gastrointestinal zinc absorption as a confounding factor in prior studies. Once studies were selected based on the zinc lozenges that

lacked these known additives and delivered greater than 75 mg zinc per day, the meta-analysis demonstrated that the common cold duration was reduced by 33% (33).

4. The human oral mucosa and gastrointestinal tract highly expresses the ACE2 receptor, which is targeted by SARS-CoV-2 for entry into cells with help from TMPRSS2.

Recently, Hoffman M., et al. 2020 demonstrated that ACE2 (angiotensin-converting enzyme 2) is the viral target receptor for SARS-CoV-2 with the help of TMPRSS2 (transmembrane Serine Protease 2) which cleaves part of the viral spike protein (34, 35). Also, Xu X., et al., 2020 has modeled the structural interaction between ACE2 and the viral spike protein (36), which has been now validated with a crystal structure of the complex (37). Therefore, cells that express both ACE2 and TMPRSS2 are prime targets for infection by SARS-CoV-2.

Xu H. et al., 2020 used a gene expression analysis of TCGA (The Cancer Genome Atlas) and FANTOM5 CAGE (Functional ANnoTation Of the Mammalian genome 5 Cap Analysis of Gene Expression) to show that the human oral mucosa expresses *ACE2* (38). Likewise, a search of the Human Protein Atlas (HPA) (39, 40) for *ACE2* tissue expression showed the highest gene and protein expression of ACE2 is in the gastrointestinal tract (41). In addition, an HPA search showed the highest gene expression and one of the highest protein expressions of TMPRSS2 in the gastrointestinal tract (42). Together, this strongly suggests that the oral mucosa and possibly the whole gastrointestinal track are the first sites of SARS-CoV-2 infection and that the lung epithelium is primarily a second site of infection. This is corroborated by gastrointestinal symptoms and loss of taste being early symptoms in COVID-19 (43, 44). Also, saliva is more sensitive than nasopharyngeal swabs in detecting SARS-CoV-2 in a recently demonstrated study (45). So far, three specific cell types have been identified as co-expressing ACE2 and TMPRSS2: i) ileal absorptive enterocytes, ii) nasal oblet secretory cells,

and iii) lung type II pneumocytes (46). Moreover, the oral mucosa and gastrointestinal track as primary first sites of infection would explain why many infected individuals are asymptomatic early on with respect to the more dangerous respiratory symptoms. This line of reasoning provides a basis for why oral administration of zinc could be an effective treatment for reducing the severity of COVID-19.

5. A recent retrospective observational study demonstrates zinc's efficacy in treating COVID-19.

In New York City, NY USA, a recent retrospective observational study compared COVID-19 patient outcomes given hydroxychloroquine plus azithromycin, with and without zinc sulfate (47). The zinc sulfate, hydroxychloroquine, and azithromycin group included 411 patients and the hydroxychloroquine and azithromycin only group included 521 patients, all admitted between March 2 and April 5, 2020. While the addition of zinc sulfate did not reduce length of hospitalization, duration of ventilation, or ICU duration, zinc sulfate did increase the frequency of patients being discharged home and reduced mortality or transfer to hospice for patients who were never admitted to the ICU. These results stayed statistically significant (p-value < 0.01), even after controlling for when zinc sulfate started being used in treatment (March 25). While this study was not a randomized clinical trial, these results do support the presented hypothesis.

6. Summary of evidence supporting the hypothesis.

Starting with the first group of evidence, high intracellular zinc concentrations are able to inhibit the SARS-CoV RDRP-RTC and given the high sequence and structural similarity between SARS-CoV RDRP machinery and SARS-CoV-2 RDRP-RTC, zinc is highly likely to inhibit SARS-CoV-2 RDRP-RTC. This is further supported by the meta-analysis of zinc lozenge studies (third group of evidence) that demonstrate a reduction in the duration of the common

cold and by the reduced mortality and transfer to hospice for COVID-19 patients treated with a combination of zinc sulfate, hydroxychloroquine, and azithromycin (fifth group of evidence). Furthermore, zinc is orally and gastrointestinally absorbed (second group of evidence), especially with high dosage. The oral mucosa and gastrointestinal tract have the highest expression of ACE2 and TMPRSS2, which are required for SARS-CoV-2 infection (fourth group of evidence). Furthermore, the oral mucosa and gastrointestinal tract are likely the first primary sites of SARS-CoV-2 infection (fourth group of evidence). Therefore, one proposed mechanism of action for orally absorbed zinc is the inhibition of SARS-CoV-2 RDRP-RTC in the oral mucosa and possibly the gastrointestinal tract in order to delay the spread of the infection to the lung epithelium. A complementary mechanism is the inhibition of SARS-CoV-2 RDRP-RTC in the lung epithelium with the help of a zinc ionophore. Together, this line of reasoning supports the overall hypothesis presented.

7. Potential synergy with other drugs.

Given the proposed mechanism of inhibiting SARS-CoV-2 RDRP-RTC by high zinc concentration in the oral mucosa and possibly the gastrointestinal tract, any zinc ionophores that promote zinc absorption are likely to be synergistic with orally administered zinc in the form of a zinc lozenge. In particular, chloroquine (48), clioquinol (49-51), quercetin (51), and epigallocatechin-gallate (51) are well-documented zinc ionophores. Therefore, the proposed use of chloroquine and its derivatives in the treatment of COVID-19 may utilize zinc transport as a mechanism of action. Given the structural similarity of quinine, ferroquine, chloroquine, hydroxychloroquine, and clioquinol (see Figure 1), all of these derivatives are either known or plausible zinc ionophores. Likewise, these drugs may be synergistic with zinc lozenges via a common mechanism of zinc transport. Quinine synergy with zinc is further supported by a demonstration of a complex of zinc and quinine *in vitro* that is three times more effective at

treating malaria than quinine sulfate (52) and more effective than chloroquine (53). Also, Biot et al. 2006 demonstrated reduction of SARS-CoV replication with chloroquine and ferroquine, but not hydroxychloroquine, under similar cell culture experimental conditions (54), which in this context may indicate reduced zinc transport efficiency by hydroxychloroquine. Since chloroquine and its derivatives are systemically circulated (55-57), it is possible that the mechanism of zinc transport which promotes SARS-CoV-2 RDRP-RTC inhibition could synergistically occur in the lung epithelium as well.

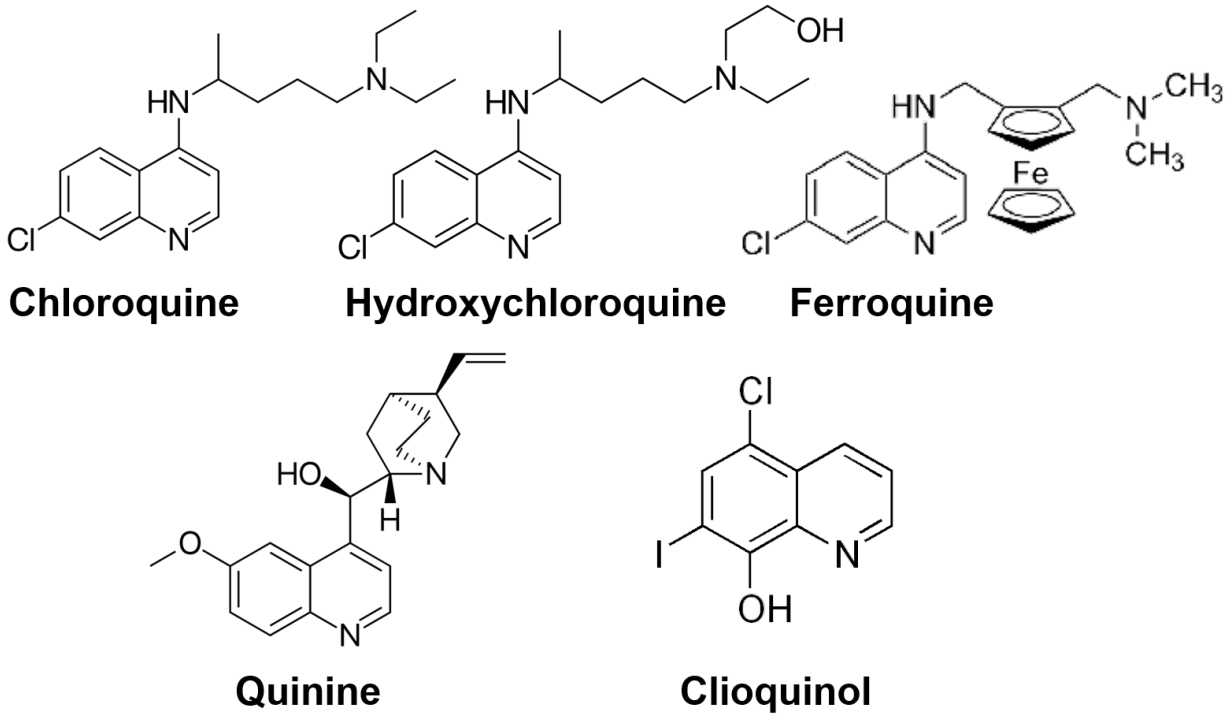

Figure 1. Chemical structure of chloroquine and similar derivatives.

8. Dietary zinc intake and zinc depleting drugs are possible confounding factors in current clinical studies involving chloroquine and its derivatives.

If chloroquine and its similar derivatives have a zinc transport mechanism in their treatment of COVID-19, then dietary zinc intake is a very likely confounding factor in current clinical studies involving these drugs. A related possible confounding factor are drugs that increase zinc

depletion (58, 59). Also, zinc absorption in humans is also reduced by other dietary factors including phytate (60-62), which can be mitigated by other dietary factors including vitamin D (63-65). Likewise, zinc absorption may be affected by age, with some studies indicating that basal zinc absorption decreases with age (66-69), while loading zinc absorption increases with age (70). However, age-related differences in plasma zinc concentrations are likely more complex due to changes in systemic zinc metabolism (70).

Dietary zinc intake and related zinc depleting drugs as confounding factors is supported in the following example of conflicting mice studies involving the treatment of coronavirus infections with chloroquine. In Barnard et al. 2006, chloroquine did not significantly reduce the replication of SARS-CoV in BALB/c mice (71), even though reduction of SARS-CoV replication *in vitro* had previously been demonstrated with chloroquine (54, 72, 73). This difference in results can be explained by the presence of zinc in the cell culture experiments, since many cell culture media contains zinc (74) and zinc is a common contamination in many labware items and water sources (75). Moreover, Keyarts et al. 2009 demonstrated chloroquine's inhibition of HCoV-OC43 replication in newborn mice (76). In this study, the newborn mice received chloroquine either transplacentally or via maternal milk. These results can be explained by placental zinc transport (77) and high zinc content (370 μM zinc) in mouse maternal milk (78).

Already there are conflicting results from published (and preprint) clinical studies on the use of hydroxychloroquine in the treatment of COVID-19 (79-82). The two studies in Marseille, France (79) and Wuhan, China (82) demonstrated a positive result. The two studies in Paris, France (80) and Shanghai, China (81) demonstrated a negative result. After a non-exhaustive search for published human zinc deficiency studies at all four locations, only Paris, France had documented nutritional zinc deficiency in infants and children specifically from low income households (83, 84) as well as homeless men (85). This mirrors a similar study illustrating

childhood zinc deficiency in low income African-American and Hispanic households in Atlanta, Georgia USA, which could partially explain the current impact of COVID-19 on these populations in the US (86). Also, the Paris, France and Shanghai, China studies involved 11 and 30 total subjects, respectively, versus 20 and 62 total subjects in Marseilles, France and Wuhan, China, respectively. All of the studies are likely underpowered and have multiple issues with their experimental design (81, 87). Without the inclusion of patient zinc plasma/tissue levels along with zinc depleting medications being taken, meta-analysis of these studies will likely have difficulties dealing with dietary zinc intake as a confounding factor. This is unfortunate, since a meta-analysis may be required to quickly generate enough statistical power out of the growing number of small underpowered clinical studies being published. Also, trying to use detailed patient diets along with the acquisition of regional zinc content of drinking water and available foods will likely prove too difficult to incorporate into meta-analyses, given the variability in food and water zinc content (88). Fortunately, a recent retrospective observational study provides some statistically significant results supporting the inclusion of zinc with hydroxychloroquine (47). There are also four clinical studies (clinicaltrials.gov IDs NCT04335084, NCT04326725, NCT04342728, and NCT04334512) underway using zinc as part of the intervention in the treatment of COVID-19; however, they will take time to produce results given that two of these trials are not even recruiting yet.

9. Possible issues with effective zinc lozenge composition and zinc dosage.

There are several additives that lower the oral and gastrointestinal absorption of zinc including: citric acid, tartaric acid , sodium bicarbonate, palm-kernel oil, cotton-seed oil, soy lecithin, mannitol, and sorbitol (33, 89-95). Also, pH affects zinc absorption in the gastrointestinal tract (96), and therefore, is likely to affect zinc absorption by the oral mucosa. Many of the over-the-

counter zinc lozenges contain one or more of these additives, especially citric acid. Also, tartaric acid is a main ingredient in cream of tartar, which is a common ingredient in many hard candy recipes, along with various fruit flavorings that contain citric acid (97). Isomalt, which is the main sugar substitute in sugar-free zinc lozenges, has a mannitol functional group. Also, Kashimura et al., 1996 demonstrated that dietary addition of isomalt reduces calcium, magnesium, and phosphorus absorption, which is rescued by the dietary addition of acetic acid (98). Given the chemical similarities between calcium, magnesium, and zinc, isomalt is likely to have a similar repressive effect on zinc absorption.

Given all of the additives that can reduce oral and gastrointestinal absorption of zinc, it is non-trivial to obtain an over-the-counter zinc lozenge that is maximally absorptive in the oral cavity and the gastrointestinal tract. An alternative is to develop a hard candy recipe that does not include any of the additives that would reduce zinc absorption. A simple recipe of zinc gluconate, sugar, light corn syrup, and water was straight-forward to develop, but requires alterations from classical hard candy recipes (99). However, a sugar-free recipe is another story. Isomalt is the classic sugar-substitute used in almost all commercially-available hard candies. There are virtually no sugar-free hard candy recipes described online. Inulin is a possible substitute, based on Mineo et al., 2004 and Coudray et al., 2006, which provided evidence from rat studies that zinc gastrointestinal absorption of zinc is enhanced by the difructose anhydrides present in inulin (100, 101). In combination with stevia as a sugar-substitute, a recipe of zinc gluconate, inulin, stevia, and water does produce a zinc lozenge, but requires freezing during the cooling process, since the lozenge never reaches a hard crack candy state (99). It is unclear if such a recipe is adaptable to hard candy manufacturing.

Zinc has an estimated human toxicity of 27 g zinc per day, but can become emetic (causing vomiting) at doses above 225 mg per day (102). Also, zinc lozenges providing doses between 75-200 mg per day for short periods of time (5 to 10 days) have shown no adverse

effects while demonstrating a reduction in duration of the common cold (33). However, the estimated IC50 (inhibition) for SARS-CoV RDRP-RTC is 1.4 μM pyrithione with 2 μM zinc in Vero-E6 cells, which is 3 times higher pyrithione than IC50 for EAV RDRP-RTC (0.5 μM pyrithione with 2 μM zinc) (1). Therefore, higher zinc lozenge doses are likely warranted in the treatment of COVID-19. However, zinc lozenges in the 200 mg per day still may not deliver a high enough dose to inhibit SARS-CoV-2 without combining it with a zinc ionophore. This may be especially true during high systemic inflammation conditions caused by injury and infection, which leads to sequestration of zinc in the liver (103-106). There is also some *in vitro* evidence that a zinc:chloroquine complex is not toxic (107) and a zinc:quinine complex is not toxic (52, 53).

3. Conclusions

A complete scientific premise involving five groups of evidence was presented supporting the hypothesis that COVID-19 severity can be reduced with the administration of zinc in an orally and gastrointestinal absorbable form. This scientific premise supports future testing of orally administered zinc in a form that maximizes oral and gastrointestinal absorption for the treatment of COVID-19. Also, dietary zinc intake and related zinc depleting drugs represent serious potential confounding factors with current clinical studies using chloroquine and similar derivatives in the treatment of COVID-19. It is recommended that the measurement of zinc in subjects be considered in the experimental designs of current and future studies.

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Abbreviations

ACE2: angiotensin-converting enzyme 2

EAV: equine arteritis virus

FANTOM5 CAGE: Functional ANnoTation Of the Mammalian genome 5 Cap Analysis of Gene Expression

HPA: Human Protein Atlas

p38 MAPK: p38 mitogen-activated protein kinase

RDRP: RNA-dependent RNA polymerase

RDRP-RTC: RNA-dependent RNA polymerase replication and transcription complex

SARS-CoV: severe acute respiratory syndrome coronavirus of the genus Betacoronavirus and causative agent of SARS

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2 of the genus Betacoronavirus and causative agent of COVID-19

TCGA: The Cancer Genome Atlas

TMPRSS2: transmembrane Serine Protease 2

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