Title:
Treatment with zinc plus resveratrol to reduce SARS-CoV-2 shedding or symptoms: a randomized, double-blind, placebo-controlled Phase 1/2 trial.

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Background Information on Zinc and Resveratrol:

The safety of high doses of zinc have been studied in the treatment a variety of metabolic conditions including Wilson's Disease[1,2]. Doses of 150 mg daily for prolonged periods of time result in depletion of copper by inhibiting intestinal copper absorption [3-6]. Sammans has shown that dosing at this level requires in excess of 6 weeks to produce copper deficiency[3]. These doses for shorter time periods have produced little toxicity other than some potential for headache, abdominal cramps, dysgeusia and nausea[4-6].

Zinc is not inherently bioavailable, making it difficult to achieve high intracellular concentrations. However, a variety of compounds have been shown to act as ionophores, increasing transport of extracellular zinc ions across cell membranes to increase intracellular zinc levels. This might potentiate its antiviral activity[7,8]. A number of zinc ionophores have been identified, including the clinically available chloroquine[9], quercetin[10] and resveratrol [11]. Chloroquine was eliminated as a candidate for this study due to its known toxicities[12]. Hydroxychloroquine was eliminated as we are unaware of direct evidence of its effectiveness as a zinc ionophore as well as its toxicities and lack of efficacy in COVID-19 trials[13-16]. Quercetin has been proposed as a potential SARS-CoV-2 antiviral[17]. In a study in Wuhan, COVID patients were given traditional Chinese medicinal remedies including herbs with high quercetin content in addition to conventional therapies. Luo reported some improvement

in symptoms with this approach[18]. However, we are unaware of any direct evidence of an antiviral effect of quercetin on SARS-CoV-2.

Resveratrol is a naturally occurring polyphenol stilbene found mostly in grapes, wine, peanuts, cocoa, and *Vaccinium* species, including blueberries, bilberries, and cranberries. It has been shown to have antioxidant and anti-inflammatory properties and has been studied as a lipid-lowering therapy, cardioprotective agent and as a potential chemopreventive for various forms of cancer[19-22].

Resveratrol and other polyphenols have been studied as possible antiviral compounds *in vitro*. Abba, et al, and others have reviewed the data showing activity against a variety of viruses including influenza virus A, respiratory syncytial virus, varicella zoster virus, Epstein-Barr virus, herpes simplex virus, human immunodeficiency virus, African swine fever virus, enterovirus, human metapneumonia virus, and duck enteritis virus[23-25]. Lin, et al have reported *in vitro* activity against MERS virus by resveratrol, though the cytotoxic effect was seen in the 125-250 uM range[26]. Recently, Yang et al have shown that resveratrol effectively inhibits COVID- 19 in Vero cells with an EC_{50} of 4.48 uM[27]. It has also been shown in animal studies that resveratrol supplementation can upregulate ACE2 receptor activity and studies have shown a correlation between loss of ACE2 receptor and increased severity of COVID[28].This has led to the hypothesis that upregulation of ACE2 with resveratrol may have a protective effect against SARS-CoV-2, though there is no specific data that we are aware of to support this theory.

Studies of benign and malignant prostate tissue have documented that intracellular zinc levels fall with the transition from benign to malignant tissue[29].

Concurrent administration of resveratrol in concentrations between 2.5 and 10uM has demonstrated progressive dose-related increases in intracellular uptake of zinc into prostate cells. This was increased with zinc supplementation concurrent with resveratrol administration, suggesting that it might well serve as a way to augment zinc uptake to potentiate its antiviral properties[30].

After oral ingestion, resveratrol is rapidly metabolized and much is excreted in urine and feces, although deconjugation of the drug *in vivo* has been reported[31,32]. Both single dose and daily dose pharmacokinetics have been studied in human[33-37]. A single dose of 5 grams resulted in peak plasma levels of approximately 2.3 uM. However, there is evidence that with repeated dosing peak plasma levels can be considerably higher[35]. At 2 grams twice daily, maximum serum concentrations of resveratrol were approximately 4 uM[33]. The major toxicity reported at this dose was loose stools. Mild nausea and lethargy were reported along with loose stools or diarrhea in a study of micronized resveratrol (SRT501) at a dose of 5 grams daily for 14 days in patients with colon cancer[38]. This study also found markedly improved absorption utilizing a micronized preparation of resveratrol as well as higher resveratrol blood levels and high concentrations of resveratrol in liver[38]. It should be noted, however, that while no other significant toxicities were observed in that study or others in which diabetics or healthy volunteers were given the same dose of SRT501, a study of this specific drug in patients with multiple myeloma was stopped because of a greater than anticipated incidence of renal failure. The conclusion of the investigators was that the drug increased the risk of dehydration, presumably from nausea, which exacerbated the severity of myeloma renal disease[39]. Mild headache has also been reported in other studies[20,33,40].

While it is unknown whether clinically achievable concentrations of resveratrol

have significant anti-SARS-CoV-2 activity *in vivo*, it seems possible that resveratrol might not only be an active antiviral but also serve as a means to increase intracellular zinc, thereby potentiating the antiviral property of zinc. It appears that 2 grams twice daily resveratrol, which has been shown to be a safe dose, can achieve plasma levels that have been shown to increase intracellular zinc levels[20,33].

References:

1. Brewer G.J.,et. al (1993). Treatment of Wilson's disease with zinc: XI. Interaction with other anticopper agents. J Amer Coll Nutr. **12,** 26-30.
2. Brewer G.J., and Yuzbasiyan-Gurkan, V. (1992). Wilson disease. Medicine. **71,** 139-164.
3. Sammans, S, and Roberts, D.C.K. (1987). The effect of zinc supplements on plasma zinc and copper levels and the reported symptoms in healthy volunteers. Med J Austria. **148,** 247-249.
4. Broun E.R., et. al. (1990). Excessive zinc ingestion. A reversible cause of sideroblastic anemia and bone marrow depression. J Amer Med Assn. **264,** 1441-1443 .
5. Hooper P.L., et. al.(1980). Zinc lowers high-density lipoprotein-cholesterol levels. J Amer Med Assn. 244(17):1960-1961.
6. Zinc Fact Sheet for Health Professionals. (2020). https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/. [Accessed July 6, 2020].
7. te Velthuis, A.J., et. al. (2010). Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture..PLoS Pathogens. **6,** e1001176 (2010). https://doi.org/10.1371/journal.ppat.1001176.
8. Ishido, T. (2019). Review on the role of Zn^{++} Ions in Viral Pathogenesis and the Effect of Zn^{++} Ions for host cell virus growth inhibition. Amer J Biomed Sci & Res **2:**28-37.
9. Xue, J., et. al. (2014). Chloroquine is a zinc ionophore..*PloS One.* **9,** e109180 (2014). https://doi.org/10.1371/journal.pone.0109180.
10. Dabbagh-Bazarbachi, H, et al. (2014). Zinc ionophoreactivity of quercetin and epigallocatechin-gallate: From Hepa 1-6 cells to a lioposome model. J Agricult Food Chem **62,** 8085-8093.
11. Zhang J.J., et. al.(2009). Effect of resveratrol and zinc on intracellular zinc status in normal human prostate epithelial cells. Amer J Physiol Cell Physiol. **297,** C632-644.
12. Della Porta, A., et al. (2020). Acute chloroquine and hydroxychloroquine toxicity: A review for emergency physicians. Am J Emer Med **38,** 2209-2217.
13. Geleris, J., et al. (2020). Observational study of hydroxychloroquine in hospitalized patients with COVID-19. N Eng J Med 382**,** 2411-2418.
14. Magagnoli, J., et al. (2020). Outcomes of hydrooxychloroquine usage in United Sttes veterans hosiptalized with COVID-19. J Am Med Assn **323,** 2493-2502.
15. Cavalcanti, A.B., et al. (2020). Hydroxychloroquine with ore without azithromycin in mild-to-moderate Covid-19. N Eng J Med **383,** 2041-2052.
16. RECOVERY Collaborative Group. (2020). Effect of hydroxychloroquine in hospitalized patients with Covid-19. N Eng J Med **383,** 2030-2040.
17. Derosa, G.,et. al. (2021). A role for quercetin in coronavirus disease 2019 (COVID-19). Phytother Res. **35,** 1230-1236.
18. Luo, E, et al. (2020).Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19). An empirical study from Wuhan, Hubei Province, China. Chinese Med **15,** 34.

19. Vang O., et al.(2011). What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. PloS One. **6,** 1-11. e19881 (2011).
20. Zhang, H., et. al. (2009). Resveratrol improves endothelial function: role of TNF{alpha} and vascular oxidative stress. Aterioscler Thromb Vasc Biol. **29,** 1164-1171.
21. Tomé-Carneiro, J., et. al. (2013). Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des. **19,** 6064-6093.
22. Singh C.K., et. al. (2013). Resveratrol-based combinatorial strategies for cancer management. Ann NY Acad Sci. **1290,** 113-121.
23. Abba ,Y., et. al. (2015). Antiviral activity of resveratrol against human and animal viruses. Adv Virol. **2015,**184241.
24. Levy, E., , V, et al. (2020). Can phytotherapy with polyphenols serve as a powerful approach for the prevention and therapy of novel coronoavirus disease 2019 (COVID-19)? Am J Phsyiol Endocrinol Metab. **319,** E689-E706 (2020).
25. Limanaqi, F., et al. (2020). Cell clearing systems as targets of polyphenols in viral infections: potential implications for COVID-19 pathogenesis. Antioxidants. **9,** 1105-1139.
26. Lin S.C., et. al. (2017). Effective inhibition of MERS-CoV infection by resveratrol. BMC Infect Dis. **17,** 144-153.
27. Yang, M., et al. (2020). Resveratrol inhibits the replication of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) in cultured Vero cells. Phyto Ther Res. **2020,** Nov 22:10.1002/ptr.69`6916 (2020). doi 10.1002/ptr.6916
28. Horne, J.R., and Vohl, M.C. (2020). Biological plausibility for interactions between dietary fat, resveratrol, ACE2, and SARS-CoV illness severity. Amer J Physiol Endo Metab. **318,** e830-e833 (2020).
29. Singh, C.K., et. al. (2014). Resveratrol-zinc combination for prostate cancer management. Cell cycle (Georgetown, Tex.). **13,** 1867-1874.
30. Zhang, J.J., et al. (2009). Effect of resveratrol and zinc on intracellular zinc status in normal human prostate epithelial cells. Amer J Physiol Cell Physiol. **297,** C632-644 (2009).
31. Cottart, C.H., et. al. (2010). Resveratrol bioavailability and toxicity in humans. Molec Nutr Food Res. **54,** 7-16.
32. Wenzel, E. and Somoza, V. (2005). Metabolism and bioavailability of trans-resveratrol. Molec Nutr Food Res. **49,** 472-481.
33. la Porte, C., et al. (2010). Steady-state pharmacokinetics and tolerability of trans-resveratrol 2000 mg twice daily with food, quercetin and alcohol (ethanol) in healthy human subjects. Clin Pharmacokin. **49,** 449-454.
34. Poulsen, M.M., et al. (2013). High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes. **62,** 1186-1195.
35. Gescher, A., et. al. (2013). Resveratrol in the management of human cancer: how strong is the clinical evidence? Ann NY Acad Sci. **1290,**12-20.
36. Boocock D.J., et al. (2007). Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. Can Epid,Biomark Prev. **16,** 1246-1252.
37. Brown, V.A., et al. (2010). Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. Can Res. **70,** 9003-9011.
38. Howells, L.M., et al. (2011). Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases--safety, pharmacokinetics, and pharmacodynamics. Can Prev Res. **4,** 1419-1425.
39. Popat, R., et al. (2013). A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. Brit J Haematol. **160,** 714-717.
40. Shaito, A., et al. (2020). Potential adverse effects of resveratrol: a literature review. Intl J Molec Sci. **21,** 2084-2109.