Improving the efficacy of chloroquine and hydroxychloroquine against SARS-CoV-2 may require zinc additives - A better synergy for future COVID-19 clinical trials

Mujeeb Olushola Shittu, Olufemi Ifeoluwa Afolami

Biological Science Department, Michigan Technological University, Houghton, Michigan, United States of America

SUMMARY

The recent outbreak of coronavirus disease 2019 (COVID-19), has now been officially declared as a pandemic by the World Health Organization. As of now, there is no known effective pharmaceutical agent against the SARS-CoV-2 virus. However, several precautionary measures have been prescribed to prevent further spread of the virus, which include avoidance of social gatherings, proper handwashing, frequently disinfecting of used items and surfaces and so on. More recent studies have highlighted the possibility of treating patients infected with the novel SARS-CoV-2 virus with chloroquine and hydroxychloroquine, of which mechanism of action is not completely understood. We seek to draw the attention of the scientific community to the possibility of drastically reducing the effects of the virus on the affected patients and improving clinical trials outcome through the synergistic action of zinc and chloroquine in patients suffering from the coronavirus disease.

Keywords: coronavirus, COVID-19, chloroquine, hydroxychloroquine, zinc, SARS-CoV-2.

INTRODUCTION

The coronavirus disease named COVID-19 by the World Health Organization, which originated from Wuhan, the capital city of Hubei province in China in December 2019 has sporadically spread throughout the world. As of today, the 16th of April 2020, over 2 million cases and 134,000 deaths have been reported in 210 countries and territories around the world [1]. The total number of cases in the United States, Spain, Italy, Germany, and France have surpassed the cases in China where the infection was originally discovered. Currently, comparative genomics studies have been deployed by some countries in Europe and North America to trace the origin of SARS-CoV-2 and to understand its evolution for proper monitoring of multiple aspects of this pandemic [2]. The infection is currently constituting a serious health, economic, social, and psychological effects on the whole world as the world is under lock down as a measure to curb the spread of the virus.

Coronaviruses (CoVs) belong to the family of *Coronaviridae*. They have a non-segmented, single-stranded, positive-sense RNA genome [3]. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19 is a zoonotic pathogen, which can infect both human and animal. This virus is believed to have crossed the species barrier to infect humans [3]. It has been

Corresponding author
Mujeeb Olushola Shittu
E-mail: mshittu@mtu.edu

suggested that human contract the SARS-CoV-2 through close contact with the animals, but there is also the possibility of foodborne transmission [4]. COVID-19 is thought to spread from person to person through respiratory droplets produced when an infected person coughs or sneezes within a proximity to an uninfected person, usually within 6 feet. Another way of spreading the virus is by touching your mouth, nose, or eyes after touching a surface or object that has the virus.

This virus infects the host cell through a non-pH dependent endocytosis by attaching to the type I integral membrane receptor angiotensin-converting enzyme-2 (ACE2) in the alveolar cells in the lungs with its glycoproteins [5]. Patients affected with SARS-CoV-2 may progress from the asymptomatic state to Acute Respiratory Distress Syndrome (ARDS) and septic shock in severe form of the disease. The common clinical features of COVID-19 include cough, sore throat, fatigue, headache, myalgia, dyspnea, and fever (not in all cases) [6].

Currently, there is no proven treatment for COVID-19 infection. However, there is a growing evidence that chloroquine and hydroxychloroquine broadly used as antimalarial and immunomodulatory drugs can be used in the treatment of patients with COVID-19 infection. Chloroquine and hydroxychloroquine belong to the same molecular family. The difference between the two is the presence of hydroxyl group at the end of the side chain of hydroxychloroquine. They are both active against malaria parasite, but hydroxychloroquine is less toxic [7]. Several *in vitro* studies and recent clinical trials have shown the efficacy of chloroquine in patients with COVID-19 at different levels of severity [8-10]. In a recent report, chloroquine was cited as a potential remedy to alleviate exacerbation of pneumonia and mitigate inflammatory response, which improves the disease outcome [9]. This is not the first-time chloroquine and hydroxychloroquine are being used to treat a novel emerged virus, there are evidences for the activities of chloroquine and hydroxychloroquine against Zika virus, Ebola virus, and Chikungunya virus [11-13]. Nevertheless, the mechanism of action of chloroquine on COVID-19 is not yet fully understood. However, several putative mechanisms describing the effects of chloroquine on the replication cycle of SARS-CoV-2 have been reported [7, 14].

Zinc is another substance that could reduce the SARS-CoV-2 viral activities when consumed due to its antiviral effect and perhaps alleviate the respiratory tract infection. Zinc is the second most abundant trace element, which exists in the divalent cation state in the body. Only a little free zinc exists because it readily binds to protein to form a metalloprotein. The primary source of zinc is a diet rich in fish, eggs, dairy products, shellfish (especially oysters), and red meat. In human, zinc supplementation is the key to constant supply of zinc and maintaining homeostasis as the ability of the body to store zinc is limited [15]. Zinc plays important roles in immunity and viral infection. Replication of SARS-coronavirus, hepatitis C virus, H1N1 influenza virus has been shown to be inhibited by zinc oxide and zinc salt. How zinc exhibits its antiviral activities is not clearly understood, however, among the possible means is the inhibition of viral binding to the mucosa, suppression of inflammatory effect, generation of antiviral interferon and inhibition of important enzyme in viral replication [16]. Recently, a study conducted by Kaushik et al. unraveled the ability of zinc salts in inhibiting Hepatitis E virus replication through the inhibition of RNA-dependent-RNA-polymerase (RdRp) [17]. Interestingly, this enzyme also plays a key role in coronavirus replication. Therefore, in this article, we will be reviewing the interaction between chloroquine, hydroxychloroquine, and zinc, and the possibility of their synergistic administration to mitigate the exacerbation of COVID-19.

Metal ionophores: their mechanistic interaction with viral replication and disease progression

Accumulated evidences in past studies have revealed that metal ionophores are drug compounds that have metal-binding domains which enable them to act as transporters of cations such as Ca^{2+}, Zn^{2+}, Na^{+} and Cu^{2+} [18-20]. Metal ions act as ligands that catalyze many downstream roles which promotes many key cellular processes. Deficiencies in concentration of metal ions like zinc, calcium or iron will significantly alter cellular signal transduction, DNA synthesis and mRNA transcription, protein aggregation and protein function [21, 22]. The ability of metal ionophores to reduce metal ion availability in extracellular matrix (ECM) of living tissues allow them to move excess ions into the cytosol thereby

affecting signal transduction [18, 23]. Drug compounds such as clioquinol, pyrithione (PT), hydroxyquinoline, chloroquine (CQ) and hydroxychloroquine (CQ) have been described as metal ionophores which can transport ion ligands that drive down stream cell signaling processes from the ECM into the cell in large amounts [23, 24]. Clioquinol and hydroxyquinoline can bind and transport Zn^{2+} and Cu^{2+} ions into cancer cells that express excess glucose receptors, causing severe metal ion toxicities and triggering the apoptotic program [25]. Similarly, metal ionophores act as weak bases and can bind excess zinc salts in viral transfected tissues and then directly interfere in synthesis of viral DNA dependent DNA polymerase or RNA dependent RNA polymerase [26]. Conversely, certain metal ionophores such as clioquinol can increase the levels of intracellular zinc in the lysosomes of cancer cells leading to lysosome-mediated apoptosis [21].

Metal ionophores may also act as chelators; a clinical trial investigation showed that drug compounds such as desferrioxamine and tetrathiomolybdate suppressed tumor clonal expansion, metastases and angiogenesis [19]. Meanwhile, clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) can inactivate superoxide dismutase-1 (SOD1) and precipitate halt in cancer progression [25]. Similarly, Daniel et al., showed that dithiocarbamate requires zinc metal ions to inhibit NF-kappa B [27]. They study also showed that zinc ions are need for PT to cause a 10-fold potency for inhibition of NF-kappa B. The zinc ionophore PT (1-hydroxypyridine-2-thionine) has been described to have antiviral properties and has been proven a potent industrial biocide [27]. In 2009, Ding and Lund reported that with adequate zinc additives, PT when added to cells along with induced apoptosis and that a zinc additive-PT treatment of viral transfected cells can represent a good frontier for clinical trial of antiviral drugs [21]. Furthermore, a later study demonstrated that novel fluorinated 8-hydroxyquinoline based metal ionophore showed potency for amyloid-beta (Aβ) deposition and stabilization in Alzheimer's disease (AD) [20]. Summarily, metal ionophores have been proven over the years to exert overt antiviral and anticancer properties especially when coupled with enough doses of metal ion additives that will galvanize their functions in living tissues.

Chloroquine and hydroxychloroquine as zinc ionophores: indirect interaction with COVID-19 genome replication

Chloroquine (CQ) is a 4-aminoquinoline antimalaria drug that has also been used over the years as an anti-inflammatory agent and as an anticancer drug [28, 29]. CQ and its derivative hydroxychloroquine (HCQ) act as weak bases that can target key cellular signal transduction organelles such as lysosomes and Golgi [30, 31]. An accumulated concentration of CQ in these organelles will catalyze significant disruption of downstream signaling processes via increase in the endosomal and lysosomal pH [28, 31]. Although, continuous study on the putative mechanism of action of CQ are still ongoing in molecular medicine, however, past studies showed that upon administration, the bioavailability of CQ and HCQ hinges largely upon their protonation with zinc ions (Zn^{2+}) upon the cell, which makes them have high affinity for low-pH organelles [32, 33]. By catalyzing an increase in the pH, CQ and HCQ impair maturation of cell lysosomes and autophagosomes, thereby inhibiting antigen presentation tendency of the host cell [31, 34]. This direct interference with lysosomal activity upon inhibition triggers an immunostimulatory response against the host cell via MHC class II presentation [33, 34]. Xue et al. showed that CQ and HCQ are zinc ionophores using human ovarian cancer cell line (A2780) [33]. They reported that at dose dependent concentrations, CQ and HCQ enhanced Zn^{2+} uptake by TPEN attenuated A2780 cells in a concentration-dependent manner. Furthermore, microscopic probe of intracellular zinc distribution demonstrated that consistent with previous studies, CQ and HCQ delivered free Zn^{2+} ions to the lysosomes inhibiting lysosomal function. The same study also suggested that a combination of CQ or HCQ with zinc enhanced chloroquine's cytotoxicity and induced apoptosis in A2780 cells [33].

Meanwhile, a study by te Velthuis et al. links an increase in the intracellular Zn^{2+} ion concentration by PT with replication impairments in RNA dependent RNA polymerase viruses such as poliovirus and influenza virus [35]. In the same study, the potency of PT zinc ionophore against these viruses was attributed to interference with polyprotein processing of RNA viruses. Meanwhile the same study also demonstrated that a combination of PT zinc ionophore with Zn^{2+} ions inhibited the

replication of RNA dependent RNA polymerase viruses; SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture. The RNA-dependent RNA polymerase (RdRp) is a core enzyme of RNA viruses that enable multiprotein replication and transcription complex (RTC) formation [35]. The same study by te Velthuis et al. used an activity assay procedure to show that without Zn^{2+}, PT was unable to effectively hinder (90%) the RNA-synthesizing activity of the RTCs of both SARS-CoV or EAV viruses [35]. They also reported further that enzymatic studies using recombinant RdRps of SARS-CoV nsp12 and EAV nsp9 showed that PT was only a transporter and that Zn^{2+} directly inhibited the activity of their polymerases *in vitro*. Hence, while PT was an ionophore that carried Zn^{2+} into the cell, Zn^{2+} acted to block the initiation of EAV RNA synthesis and SARS-CoV RdRp elongation was inhibited so that RNA template binding was reduced. Conversely, another study by Kaushik et al. investigated the effect of zinc salts on RNA replication of hepatitis E virus (HEV) using hepatoma cell (Huh7) cultures [17]. It was reported that zinc salts transported by PT inhibited the RNA replication of g-3 HEV replicons and g-1 HEV infectious genomic RNA in a dose-dependent manner [17]. Analysis of a replication-defective mutant of g-1 HEV genomic RNA showed that zinc salts directly inhibit the activity of viral RdRp, leading to inhibition of viral replication [17]. In summary, zinc ionophores such as CQ, PT and HCQ have demonstrated promising prospects for successful clinical trials by *in vivo* and *in vitro* studies where their administration is coupled with zinc supplements.

Combining CQ and HCQ use with zinc supplements: synergism needed for successful COVID-19 clinical trials?

A variety of compelling evidences have been published from early clinical trials in China that showed the efficacy of CQ and HCQ in the treatment of SARS-CoV-2. The long trail of studies showed the possibility that CQ and its derivatives may be effective against the novel SARS-CoV-2 (the pathogen that causes COVID-19 and shares a close phylogeny with previous species of coronavirus) [8, 10, 36, 37]. A common consensus amongst the published clinical trials was that the SARS-CoV-2 virus requires acidification of endosomes and that essential modifications to its capsid envelope glycoproteins are needed for viral replication which occurs within the endoplasmic and trans-Golgi network vesicles at a low pH in presence of proteases and glycosyl-transferases [8, 37-39]. However, this essential prerequisite for SARS-CoV-2 replication is blocked by CQ and HCQ since the drugs alter ACE2 glycosylation by stopping S-protein binding, thereby interfering with viral replication the cell cytoplasm [9].

Meanwhile, another recent systematic review on the state of CQ and HCQ clinical trials for COVID-19 used PubMed and EMBASE databases from inception to 1-March-2020 to find information on the efficacy and safety of CQ/HCQ formulations in patients diagnosed with SARS-CoV-2 [40]. Their initial search identified 234 sources (156 from PubMed, 73 EMBASE and 5 from other verified sources) amongst which twenty-three clinical trials were found in the trial registries. However, in all these documented clinical trials in Europe and China, the pattern of administration was similar as CQ and HCQ drugs were used without being combined with zinc ion supplements. Incidentally, none of these clinical trials conducted so far has given a near total positive outcome, which is significant enough to trigger an endorsement on a global scale. Perhaps, consistent with previous studies that delineated the efficacy of HCQ and CQ as zinc ionophores, it was rather surprising that none of these clinical trials so far considered using a combination of dose depended zinc supplements with HCQ and CQ administration.

CONCLUSION

Chloroquine can induce the uptake of zinc into the cytosol of the cell, which is capable of inhibiting RNA-dependent RNA polymerase and ultimately halting the replication of coronavirus in the host cell. Currently, there are several clinical trials that are currently underway in several countries of the world to assess the efficacy of chloroquine as an anti-coronavirus agent. Since chloroquine has been widely prescribed for use as an anti-malarial, its safety is not in doubt. In view of the foregoing, clinical trials predicated upon a synergistic administration of Zn supplement with CQ or HCQ against the novel SARS-CoV-2 virus should be considered so that better COVID-19 clinical trial outcomes can be obtained going forward.

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