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A possible application of hinokitiol as a natural zinc ionophore and anti-infective agent for the prevention and treatment of COVID-19 and viral infections

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ABSTRACT

Zinc and the combination with zinc ionophore have been reported in basic research and several clinical investigations as a potentially viable and economical preventive and therapeutic options for COVID-19 treatment. Zinc is a vital microelement that actively supports respiratory epithelium barrier integrity, innate and adaptive immune functions, and inflammatory regulations. Moreover, zinc may also prevent viral entry, suppress viral replication, and mitigate the damages due to oxidative stress and hyperinflammatory reaction in patients with respiratory infections. Hinokitiol (β-thujaplicin) is a natural monoterpenoid and is considered as a safe zinc ionophore to help zinc transport into cells. It has been widely used in skin and oral care, and therapeutic products for its potent antiviral, antimicrobial, antifungal, anti-inflammatory, and anticancer applications.

The ongoing COVID-19 pandemic and the significant morbidity and mortality exist in the high-risk group of patients associated with other respiratory infections such as influenza, respiratory syncytial virus, and dengue fever. There is an urgent need for the development of inexpensive, safe, and effective therapeutics to prevent and treat these viral infections. Considering that hydroxychloroquine (HCQ), the most studied zinc ionophore drug for COVID-19, is linked to potentially serious side effects, we propose the implementation of hinokitiol as a zinc ionophore and anti-infective agent for the prevention and treatment of COVID-19 and other viral infections.

Background to hypothesis

The World Health Organization (WHO) assumes that at least one-third of the world's population is affected by zinc deficiency [1]. Furthermore, zinc deficiency is responsible for approximately 16% of all respiratory infections worldwide [2]. This suggests potential benefits of zinc supplementation for the treatment and prevention of respiratory infections, including COVID-19.

Ionophore is a fat-soluble substance that can transport non-fat soluble elements across the cell membrane. Zinc-ionophores shuttle free zinc in or out of cells, depending on the free zinc concentration gradient, and also serve as intracellular zinc transporters for zinc compartmentation to enhance the zinc-dependent effects. An iincreasee in intracellular zinc ion and the administration of zinc ionophores have been proved to impair the replication of a wide range of RNA viruses, including rhinoviruses, influenza, coxsackievirus, mengovirus, picornavirus, herpes, and coronaviruses [3].

Hinokitiol (β-thujaplicin) is a naturally occurring monoterpenoid found in the wooden part of trees in the Cupressaceae family. It is a tropolone derivative and is widely used in oral care and therapeutic products for its potent, broad-spectrum antiviral, antimicrobial [4], antifungal, anti-inflammatory [5,6], and anticancer properties [7]. Additionally, it has been approved as a food additive and does not accumulate in the body. It has no allergic, toxic, and unfavorable effects recorded in the published literature throughout years of applications [4]. Hinokitiol is regarded as a safe zinc ionophore to help zinc influx into cells to increase the intracellular pool of labile zinc [8].

Statement of hypothesis

We propose and hypothesize that hinokitiol, as a natural and harmless zinc ionophore and anti-infective agent, can be used as a single agent or ideally in combination with an organic or inorganic zinc compound for the treatment and prevention of COVID-19 and other viral infections.

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Evaluation and discussion of hypothesis

Antiviral properties of zinc ionophore

The antiviral activity of zinc depends on the intracellular availability of zinc. Intracellular Zn^{2+} homeostasis is achieved by the opposing actions of two families of zinc transporters, ZIP involving in zinc uptake and ZnT mediating intracellular zinc trafficking and efflux [9,10]. The intracellular free Zn^{2+} concentration is maintained at a relatively low level by metallothioneins [11], and it can serve as modulators of signal transduction [12]. Zinc has direct antiviral properties that include the stimulation of a variety of antiviral signaling events, including viral polymerase function, viral protein production and processing, and viral inactivation [13,14]. For example, in vitro, a dose of zinc greater than 2 μM inhibited SARS-CoV replication by inhibiting RNA polymerase. For the herpes virus, zinc inhibitory activity has been attributed to reducing NF-κB function by interfering with the protein ubiquitination pathway [15]. Clinical studies using zinc supplements have been carried out in 'common cold" viruses such as influenza and coronaviruses. The amount of ionic zinc present at the infection sites is highly correlated to the study outcome, and is dependent on the zinc formulation [16,17].

Zinc ionophores can help transport zinc ions across the lipid membrane in the cell. This influx of zinc ions has been demonstrated in a zinc ionophore dose-dependent manner. In cell culture studies, the addition of zinc ionophores, such as hinokitiol, pyrrolidine, dithiocarbamate and pyrithione, stimulated zinc import [18,19]. The combined effects of zinc and zinc ionophores were found to inhibit the replication of various RNA viruses, including influenza virus, respiratory syncytial virus, and several picornaviruses [8,20 15,21]. Zn^{2+} and pyrithione at low concentrations inhibit the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus [22].

The antimalarial drug chloroquine (CQ) and its metabolite hydroxychloroquine (HCQ) are currently being tested in several clinical studies as promising candidates to limit SARS-CoV-2-mediated morbidity and mortality [23]. CQ is not only known as a zinc ionophore [24] to transport zinc inside cells, but it is also as an autophagy inhibitor by blocking lysosomal acidification. Furthermore, CQ carriers free zinc in the lysosome, leading to the disruption of lysosome integrity [25]. CQ inhibits pH-dependent SARS-CoV-2 replication and interferes with the delivery of virus particles into host cells. With the assistant of CQ, higher intracellular zinc levels might result in a more efficient inhibition on RNA dependent RNA polymerase (RdRp). Consequently, the effective inhibition of intracellular SARS-CoV-2 replication may potentially result in better clinical outcomes of COVID-19 patients treated with CQ or HCQ [26,27].

Hinokitiol is a safe and viable zinc ionophore for the treatment and prevention of COVID-19 and other viral infections

Hinokitiol, a tropolone-related compound found in heartwood cupressaceous plants, exhibits multiple biological activities such as anti-inflammatory, antitumorigenic, antiviral, antifungal, antimicrobial and antioxidants activities [5,28,29]. Hinokitiol was discovered in Japan in 1936 [30]. It has been broadly used in consumer oral care products and as an antimicrobial hand-washing solution [4]. Early clinical uses of hinokitiol consisted of treatments for lung gangrene, tuberculous fistula, pulmonary tuberculosis, and decubitus ulcers. Because of its low toxicity compared to other zinc ionophores, Hinokitiol has been applied in consumer oral care products without restriction in both the EU and the United States. It is also an approved food additive in Japan since 1956.

Hinokitiol treatments cause a rapid and efficient influx of Zn^{2+} into cells. The presence of Hinokitiol facilitated a threefold increase of intracellular Zn^{2+} within a few minutes. Hinokitiol inhibits viral replication by impairing viral polyprotein processing; however, this capability depends on the availability of zinc ions. Thus, an increase in intracellular zinc levels provides the basis for a new antipicornavirus mechanism [8]. Other studies indicated that a prolonged presence of zinc and its ionophores during viral replication is needed to exert antiviral effects against several human viruses, such as influenza, rhinovirus, coxsackievirus, herpes simplex virus, hepatitis E virus and mengovirus [5,28,29].

Other anti-infective properties of Hinokitiol

Hinokitiol has potent antimicrobial activity against many bacteria and fungi, including antibiotic-resistant pathogens [31,32]. It inhibited the growth of common human pathogens such as *Escherichia coli, Streptococcus pneumonia, Streptococcus mutans,* and *Staphylococcus Aureus* when inoculated these bacteria in optimal growth conditions [33]. The effective amount (50 μg/ml) was only one 20th against the dose to show the bactericidal activity, which was the same dose recommended for fragrance usage [34]. Hinokitiol has been proved to have inhibitory effects on Chlamydia trachomatis and may be clinically beneficial as a topical drug [35]. Low dose of hinokitiol (200 μM) has substantial antimicrobial and cytotoxic activities against oral pathogens and oral squamous cell lines and has no cytotoxic effect on normal human cells, indicating the potential for applications in oral health care [32,36]. Hinokitiol has a damaging effect in vitro on S. mansoni cercariae in a concentration- and time-dependent by preventing the cercariae from the penetrating the host skin [37]. Apart from anti-microbial effects, various bioactivities of hinokitiol have been reported, such as repellent activity for ticks, the cytotoxic effect on tumor cells and lymphocyte blastogenesis.

Hinokitiol is generally considered safe. No developmental toxicity or carcinogenic effects was observed [38,39].

Conclusion

The evaluation of existing published scientific evidence as well as the good safety profile of both zinc and hinokitiol in therapeutic, nutritional and cosmetic products imply that the composition of these two readily available and inexpensive substances can be immediately implemented as a preventive and therapeutic option for COVID-19 and other viral infections. Apart from its direct antiviral effect, hinokiotiol, as a single ingredient or preferably in combination with zinc compound, can provide anti-inflammatory, antibacterial, and antifungal activities that are frequently associated with the complicated cases of the diseases with high morbidity and mortality.

We believe that continuing research for the development of optimal therapeutic formulas and the most effective methods for administration of zinc-hinokitiol composition is essential to improve treatment and prevention of the COVID-19 pandemic and other respiratory viral infections.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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