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Potential role of zinc supplementation in prophylaxis and treatment of COVID-19

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents the largest current health challenge for the society. At the moment, the therapeutic strategies to deal with this disease are only supportive. It is well known that zinc (Zn) possesses a variety of direct and indirect antiviral properties, which are realized through different mechanisms. Administration of Zn supplement has a potential to enhance antiviral immunity, both innate and humoral, and to restore depleted immune cell function or to improve normal immune cell function, in particular in immunocompromised or elderly patients. Zn may also act in a synergistic manner when co-administered with the standard antiviral therapy, as was demonstrated in patients with hepatitis C, HIV, and SARS-CoV-1. Effectiveness of Zn against a number of viral species is mainly realized through the physical processes, such as virus attachment, infection, and uncoating. Zn may also protect or stabilize the cell membrane which could contribute to blocking of the virus entry into the cell. On the other hand, it was demonstrated that Zn may inhibit viral replication by alteration of the proteolytic processing of replicase polyproteins and RNA-dependent RNA polymerase (RdRp) in rhinoviruses, HCV, and influenza virus, and diminish the RNA-synthesizing activity of nidoviruses, for which SARS-CoV-2 belongs. Therefore, it may be hypothesized that Zn supplementation may be of potential benefit for prophylaxis and treatment of COVID-19.

Introduction

The 2019–2020 will be reminded for the worldwide pandemic of the Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease was first identified in December 2019 in Wuhan, Hubei Province, China, and owed to very high contagiosity has quickly spread globally, thus on March 11, 2020, the World Health Organization (WHO) has recognized it as pandemic. Obviously, COVID-19 represents the largest acute health challenge for the society in the modern history of mankind. According to the WHO report (March 6, 2020), the crude mortality ratio (reported deaths divided by reported cases) is 3–4%, whereas these rates vary by country, patient age, and presence of co-morbidities. Most of those who have died were elderly (about 80% of deaths were in those aged 60 and more), and 75% of them had pre-existing health problems, including cardiovascular diseases and diabetes. SARS-CoV-2 could be transmitted from human to human, and symptomatic individuals are the most frequent source of the disease. As with other respiratory pathogens, including flu and rhinovirus, the transmission of SARS-CoV-2 is believed to occur through respiratory droplets from coughing and sneezing. Primarily, the transmission of disease is facilitated when people are in close contact, but it may also spread when one touches a contaminated surface and then their face. At the moment, the therapeutic strategies to deal with the COVID-19 are only supportive, and reducing transmission in the community is the only one effective preventive measure, which presumes isolation of patients and infected individuals and careful infection control.

Background

Coronaviruses constitute the subfamily Orthocoronavirinae, within the family Coronaviridae, order Nidovirales, and realm Riboviria. These are enveloped viruses with a positive sense single-stranded RNA genome and a nucleocapsid of helical symmetry [1]. The Wuhan strain has been identified as a new strain of Betacoronavirus from group 2B with approximately 70% genetic similarity to the SARS-CoV. The virus has a 96% similarity to a bat coronavirus, thus it is widely suspected to originate from bats.

Immune response led by interferons (IFN) and cytotoxic T lymphocytes are invariably required to clear viral infections. Zinc ions

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(Zn^{2+}) are closely involved in the normal development, differentiation, and function of immune cells, thus considered critical for generating both innate and acquired (humoral) antiviral responses [2].

Zn is involved in various cellular processes and possesses a variety of direct and indirect antiviral properties. It was demonstrated that Zn deficiency is associated with reduced antibody production, affected function of the innate immune system (e.g., low natural killer cell activity), decreased cytokine production by monocytes, and the chemotaxis and oxidative burst of neutrophil granulocytes [3]. It also results in thymic atrophy, altered thymic hormones production, lymphopenia, and defective cellular- and antibody-mediated responses that lead to increased rates and duration of infection. In particular, Zn deficiency reduces the number of peripheral and thymic T cells, their proliferation in response to phytohemagglutinin, and the functions of T helpers and cytotoxic T cells. In addition, Zn deficiency acts indirectly by reducing the levels of active serum thymulin, a zinc-dependent nonapeptide hormone that regulates the differentiation of immature T cells in the thymus and the function of mature peripheral T cells [4]. On the other hand, Zn can affect several aspects of monocyte signal transduction and secretion of pro-inflammatory cytokines, and interfere with the binding of leukocyte function-associated antigen-1 to ICAM-1, thus suppressing inflammatory reaction [5].

Hypothesis. *Zn supplementation may be of potential benefit for prophylaxis and treatment of COVID-19, since it possesses a variety of direct and indirect antiviral properties, which are realized through different mechanisms.*

Antiviral immune response and role of zinc ions

Previous *in vitro* studies have demonstrated that Zn induces the production of IFN-α and IFN-γ and can potentiate the antiviral action of the former. *Ex vivo* experiments showed that Zn supplementation may improve leukocyte IFN-α production and reduce mononuclear cell tumor necrosis factor (TNF) production [3]. In healthy humans, Zn supplementation has also decreased the production of TNF-α and interleukin-1β [6]. Zn also enhances cell's resistance to apoptosis through inhibition of caspases-3, −6, and −9, and an increase of the Bcl-2/Bax ratio [7], and such antiapoptotic effects at both the peripheral and thymic level could result in an increase in the number of T helpers. Zn-induced alteration of the capillary epithelium might inhibit transcapillary movement of plasma proteins and reduce local edema, inflammation, exudation, and mucus secretion [8]. Finally, Zn may also protect or stabilize the cell membrane which could contribute to an inhibition of the entry of the virus into the cell [9].

Antiviral effects of Zn may be also realized through metallothioneins (MT), a family of low molecular weight, cysteine-rich proteins, which functions include storage and transfer of Zn^{2+}. Schoggins et al. [10] who showed that overexpression of multiple members of the MT1 family inhibits replication of flaviviruses (e.g., yellow fever virus and HCV), as well as the alphavirus (Venezuelan equine encephalitis virus). Antiviral effects of MT may be either direct with sequestering Zn^{2+} away from the viral metalloproteins or indirect by acting as Zn chaperones and facilitating antiviral signalling.

Effectiveness of zinc supplementation in viral infections

Zn deficiency is associated with increased susceptibility to infectious diseases caused by bacterial, viral, and fungal pathogens, and may be caused by some diseases (e.g., liver cirrhosis or inflammatory bowel disease), aging, and lifestyle-associated factors (e.g., vegan/vegetarian diet) [2,11]. In such cases, appropriate administration of Zn supplement in sufficient therapeutic doses has a potential either to restore depleted immune cell function or to improve normal immune cell function. It may also act in a synergistic manner when co-administered with standard antiviral therapy [2].

Zn supplementation in patients with HCV has decreased liver inflammation, enhance response to antiviral therapy, effectively inhibits production of viral oncogenic proteins E6 and E7 and helps to regain the function of tumor suppressors p53 and pRb [12]. Zn given in combination with IFN-α was more effective against chronic hepatitis C than a therapy with IFN-α alone [13]. Topical Zn formulations may be helpful for treatment of vaginal HPV infections in unvaccinated women. Zn supplementation is considered as the most effective systemic treatment for viral warts [14]. A randomized trial demonstrated that Zn supplementation shortens the length of diarrhea episodes and reduced the rate of treatment failure or death by 42% in zinc-deficient children [15]. Addition of Zn supplementation to antiretroviral therapy in patients with HIV has resulted in significant increase of $CD4^+$ T cell count in comparison to control group treated with antiretroviral therapy alone [16].

Potential role of zinc supplementation against SARS-CoV-2

Antiviral properties of Zn against a number of viral species are mainly realized through the physical processes, such as virus attachment, infection, and uncoating, as well as through inhibition of viral protease and polymerase enzymatic processes [2].

Zn^{2+} are considered crucial for the proper folding and activity of various cellular enzymes and transcription factors, and may be an important co-factor for numerous viral proteins as well. Zn^{2+} may interfere with the proteolytic processing of viral polyprotein by its misfolding, direct actions on the viral protease (as in picorna virus, encephalomyocarditis virus and polio virus) and alteration of the tertiary structure (as an encephalomyocarditis virus) [17]. Zn may also efficiently inhibit membrane fusion of respiratory syncytial virus, HSV, Semliki Forest virus and sindbis viruses, which is realized through binding to a specific histidine residue revealed on the viral E1 protein at low endosomal pH [18]. Finally, Zn^{2+} have a potential for direct inactivation of the free Varicella-Zoster virus *in vitro* [19].

Cell culture studies have demonstrated that high Zn concentrations and the addition of pyrithione for stimulation of the cellular import of Zn^{2+} result in inhibition of the replication of various RNA viruses, including influenza virus, respiratory syncytial virus, and several picornaviruses [17,20]. It was suggested that in picornaviruses and coronavirus such an effect is realized due to the interference with viral polyprotein processing [21]. Viral RNA-dependent RNA polymerase (RdRp) are suitable targets for novel antiviral drugs, since their activity is strictly virus-specific and may be blocked without severely affecting key cellular functions. Of note, an inhibitory effect of Zn on function of viral RdRp was demonstrated in cases of rhinoviruses, HCV, and influenza virus [22,23]. In particular, *in vitro* studies have demonstrated that Zn salts can reduce HCV replication in E. coli by 50% (at 100 μM $ZnSO_4$) by inhibiting the HCV RdRp [24].

Nidoviruses is a large group of positive-strand RNA (+RNA) viruses, which includes major pathogens of humans and livestock, such as SARS-CoV and other human coronaviruses, the arteriviruses (e.g., equine arteritis virus [EAV]), and porcine reproductive and respiratory syndrome virus (PRRSV) [25,26]. Zn effectively inhibits the RNA-synthesizing activity of nidoviruses (including SARS-CoV) *in vitro*, which is realized through alteration of RdRp activity during the elongation phase of RNA synthesis, probably by directly affecting template binding [24]. Such an effect could be reversed by addition of a Zn^{2+} chelator (MgEDTA). Thus, it may be suggested that in coronaviruses, Zn^{2+} may inhibit both the proper proteolytic processing of replicase polyproteins and RdRp activity [24].

Of note, like other coronaviruses, SARS-CoV-2 causing COVID-19 also comes under nidovirus group. RdRp and 3CLpro protease of SARS-CoV-2 share over 95% of sequence similarity with those of SARS-CoV despite the fact that these two viruses demonstrate only 79% sequence similarity at the genome level [27]. It allows to hypothesize that antiviral effects of Zn may be realized in SARS-CoV-2 as well.

Conclusion

Zn possess several antiviral effects which are realized through the generating both innate and acquired (humoral) immune responses, facilitation of the normal functioning of innate immune system, stabilization of cell membrane inhibiting the entry of the virus, and inhibition of viral replication through interference with the viral genome transcription, protein translation, polyprotein processing, viral attachment, and uncoating. Multiple antiviral effects of Zn have been demonstrated in a variety of viral species, including several nidoviruses, for which SARS-CoV-2 belongs. It suggests that Zn supplementation may be of benefit for prophylaxis and treatment of COVID-19. Considering current absence of effective therapies for this disease, its high contagiosity, frequent life-threating course, and tremendous negative impact on the affected individuals and healthcare systems worldwide, the presented hypothesis requires urgent testing in humans.

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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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