Original Research Paper

Volume - 11 | Issue - 01 | January - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pharmacology

# ROLE OF ZINC IN COVID-19- A REVIEW

| | |
|---|---|
| **J. Jayasheela** | Assistant Professor, Department of Pharmacology, Sri Lakshmi Narayana Institute of Medical Sciences. |
| **G. Somasundaram*** | Professor and HOD, Department of Pharmacology, Sri Lakshmi Narayana Institute of Medical Sciences. *Corresponding Author |
| **Madan Shubham Sanjay** | 2nd year MBBS Student, Sri Lakshmi Narayana Institute of Medical Sciences. |
| **S. Jaikumar** | Associate Professor, Department of Pharmacology, Sri Lakshmi Narayana Institute of Medical Sciences. |

**ABSTRACT** COVID-19 belongs to β-CoV genera which primarily affects the host pneumocytes causing mild to severe symptoms ranging from non-productive (dry) cough to difficulty in breathing. Zinc a biologically stable metal belonging to first row transition elements and also an important factor for immune cells proliferation when used as a supplement along with Zn ionophores in these patients may inhibits the core enzyme- RNA dependent RNA polymerase (RdRp) activity and hence significantly hinders the replication of this virus. In course of infection the immunomodulatory role and synergistic effect of zinc with antiviral drugs could play an important role in patients affected with SARS-CoV-2.

**KEYWORDS :**

## INTRODUCTION

On March 11, 2020, the World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19) outbreak as pandemic caused by member of the Coronaviridae family—SARS-CoV-2. Coronaviruses form the subfamily Orthocoronavirinae, belong to the family Coronaviridae, order Nidovirales, and kingdom Riboviria. Coronaviruses are classified into four genera based on the genetic properties: α-CoV, β-CoV, γ-CoV, and δ-CoV[1], and the COVID-19 belongs to β-CoV[2]. Like other coronaviruses, SARS-CoV-2 is an enveloped positive single-strand RNA genome [3].

They cause respiratory tract infections in birds and humans that can range from mild to fatal. Mild infection in humans include common cold (which can also be caused by other viruses such as rhinovirus) while more dangerous varieties can cause SARS, MERS, and COVID-19.SARS-CoV-2 primarily infects alveolar cells and alveolar macrophages. SARS-CoV-2 causes an inflammatory condition also known as pneumonia affecting primarily alveoli[1]. Typically, symptoms include non-productive cough, chest pain, fever, and difficulty in breathing.

### Replication of SARS -CoV- 2 in hosts pneumocytes

Once virus entered into host cell and uncoated inside the host cell. The viral genome has been translated to large poly precursor polyproteins la (pp1a) and 1ab (pp1ab) which are processed by proteins such as ORF 1a-encoded viral proteinases, papain-like proteinase ($PL^{pro}$) and 3C-like proteinase ($3CL^{pro}$), form 16 mature nonstructural proteins (nsp1–nsp16) according to their order from N-terminus to C-terminus of ORF 1a polyproteins. The replicase proteins have functions such as the RNA-dependent RNA polymerase (RdRp; NSP12), modifying methyltransferases (NSP14 and NSP16), RNA cap- and an exoribonuclease (NSP14) and a helicase (NSP13) for viral RNA synthesis and capping. Using the host proteins, the coronavirus NSPs undergo membrane-associated replication and transcription complexes provide viral membrane structures. Detailed function of each protein is explained by Cheng et al.[4]

SARS-CoV-2 contains 16 non- structural protein (NSPs) and four major structural proteins as spike (S) protein, envelop (E) protein, membrane (M) protein and nucleocapsid (N) protein[5]. S1 and S2 are two domains of S protein responsible for receptor binding and cell membrane fusion respectively, which helps the virus to bind with cells of the host. It is noted that C-terminal of S1 contains receptor binding domain (RBD) of β-CoV[6]

The binding affinity of spike proteins of SARS-CoV-2 towards angiotensin-converting enzyme 2 (ACE2) receptors is 10 to 20 fold higher as compared to that of SARS-CoV[7]. The SARS-CoV-2 binds with angiotensin-converting enzyme 2 ACE2 receptors expressed on host pneumocytes using spike glycoproteins (S-glycoprotein)[8].

It is generally seen that on the epithelial cells of alveoli, trachea, bronchi, bronchial serous glands and alveolar monocytes and macrophages[9]ACE2 receptors are widely expressed[10].

After binding to ACE receptors conformational changes take place in the S-glycoprotein allowing cleavage by the transmembrane protease-serine 2 of the S-glycoprotein. Then through the endocytosis virus is transported into cytoplasm.

The cleavage of the S-glycoprotein occurs due to the presence of low pH inside the endosomes that favours the host protease cathepsin-L which results in the fusion of the viral envelope and endosomal phospholipidic membrane to release the positive-strand viral genomic RNA (+RNA) into the cell cytoplasm. SARS-CoV-2 genome–encoded RNA-dependent RNA polymerase (RdRp) is the *central* enzyme in the *replicative cycle* of *RNA viruses like other RNA virus.*

Initially a polyprotein precursor is formed from which the RdRp-containing subunit is cleaved proteolytically. Subsequently, the RdRp is integrated into a membrane associated viral enzyme complex that drives the synthesis of negative-strand RNA[11,12].

As described in Figure-1 Synthesis of viral mRNA takes place with the help of negative RNA strand.

SARS-CoV-2, MERS-CoV, and SARS-CoV have remarkably similar sequences and encode structurally similar RdRp [13]. Infected cells contain more +RNA strands than −RNA strands.

To favour viral subgenomic mRNA synthesis, the polycistronic ribosome machinery of the infected cell synthesizes nonstructural proteins of the SARS-CoV-2 and assembles these into the replicase-transcriptase complex.

Following replication, these enveloped proteins are translated and get integrated into the endoplasmic reticulum of the host cells and finally enter into the Golgi compartment.

Consequently, envelope proteins are incorporated during the budding step to form mature virions followed by the viral genomic RNA is packaged into the nucleocapsid. The membrane (M) protein plays a vital role in interacting with the other viral proteins during viral assembly. The newly formed viral particles are transported to the cell surface in vesicles and are released by exocytosis followed by assembly. Several detailed reviews have summarized the replication

and assembly of the virus[11,14].

In patients with severe disease compared with individuals with uncomplicated SARS high serum levels of proinflammatory cytokines [IL-1, IL-6, IL-12, interferon γ (IFNγ), and transforming growth factor-β] and chemokines (CCL2, CXCL9, CXCL10, and IL-8) were found[15].

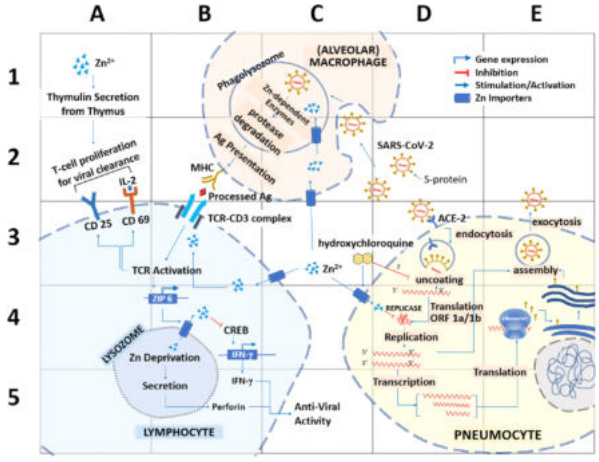


**Figure-1**[16] Possible sites of action of Zn to counter SARS-CoV-2 in pneumocytes described in Table-1 in the matrix form

**Table-1**

| | |
|---|---|
| D3 | • To bind to angiotensin-converting enzyme 2 (ACE2) on pneumocytes SARS-CoV uses spike (S) proteins<br>• Through endocytosis virus enters the host cell and releases the viral RNA |
| D4 | • The replicase enzyme complex is translated from the viral genome that mediates both replication and transcription<br>• The primary site of Zn2+ could be the inactivation of the viral replicase |
| E3 | • Virions are shed from the infected cell through exocytosis |
| B5 | • At the same time, the additional Zn supplement might initiate interferon gamma(a common anti-viral agent) production by T lymphocytes |
| (A2–3) | • T cell receptors as well as CD25 and CD69 are activated by a pool of Zn importing inside the T lymphocytes to aid T cell proliferation and stabilization |
| (A1–2) | • Added Zn also contributes to the production of thymulin from the thymus and triggers T lymphocyte production |
| (B-C2) | • In alveolar macrophages, Zn can help to degrade the phagocytosed viral particle by the enzymes of the phagolysosome |
| B2 | • That in turn will help to present the processed Ag by the major histocompatibility complex (MHC) |

Unlike other metals/elements, $Zn^{2+}$ is metal cofactor which doesn't participate in redox reactions but functions as a Lewis acid to accept a pair of electrons. Due to redox stabilization $Zn^{2+}$ is good metal for biochemical reactions and thermodynamically stable[16]. Zinc is a cofactor for the antioxidant enzyme superoxide dismutase that converts superoxide to hydrogen peroxide. It is conjointly reported that zinc deficiency may reduce the protein synthesis in taste budcells, reduce the activity of alkaline phosphatase in taste buds, alter a zinc-containing salivary protein, clog the taste pore region of the taste bud or lead to central nervous system dysfunction[17].

## Zn Catalyzes Cell-Mediated and Adaptive Immunity in the Course of Infection

Zinc acts via cell-mediated immunity against infectious agent like bacteria and virus. Zinc is one of the major sources that control function and proliferation of immune cells like neutrophils, NK cells, macrophages, B and T lymphocytes. Zn is also mediating the protection from the harmful effects of reactive oxygen species (ROS) during inflammatory processes.Free intracellular $Zn^{2+}$ is essential in extravasation to the site of the infection and uptake and killing of microorganisms by neutrophils[18].

## Immune Regulation Of Zinc In Viral Infection

The zinc-Finger CCHC- Type Containing 3 (ZCCHC3) binds RNA and facilitates the detection of intracellular RNA viruses by activating retinoic acid-inducible gene-I (RIG-1)-like receptors (RLRs), including RIG-I and melanoma differentiation- associated protein 5 (MDA5)[19]. This action triggers the activation of the antiviral response mediated by downstream activation of antiviral genes[20]. Activation of interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) take place when kinases such as TANK-binding kinase 1(TBK1) and I kappa B kinase (IκK) further phosphorylate the interferon regulatory transcription factor 3 (IRF3) and IκB-alpha, the NK-κB inhibitor which results in interferon type 1 upregulation[21,22].

Interferon alpha induced signaling results in upregulation of antiviral proteins (RNase L and protein kinase RNA-activated (PKR)), known to degrade viral RNA and inhibit its translation[23].

It was found that activation of NF-κB is inhibited by Zinc through the expression of the A20 protein. A20 may be a zinc-finger protein that negatively regulates tumor necrosis factor receptor (TNFR) and toll-like receptor (TLR)-initiated NF-κB pathways[24]. Apart from this zinc inhibits cyclic nucleotide phosphodiesterase (PDE) due to which cyclic nucleotide cGMP (cyclic guanosine monophosphate) is elevated, resulting in the activation of PKA (protein kinase A), and subsequent inhibition of NF-κB[25]. Additionally, zinc supplementation has been shown to downregulate inflammatory cytokines by decreasing gene expression of interleukin-1-beta (IL-1β), tumor necrosis factor alpha (TNF-alpha), and by inhibiting NF-κB activation[26].

Inhibition of intracellular $Zn^{2+}$ in the replicative cycle was reported in various RNA viruses including influenza virus[27] , respiratory syncytial virus[28], and several picornaviruses[29-31] as it was reported in the vitro studies compounds, such as hinokitiol (HK), pyrrolidinedithiocarbamate (PDTC), and pyrithione (PT) are zinc ionopores which transport Zn into cells.. Also specifically $Zn^{2+}$also inhibits the processing of polyprotein in cells which are infected with human rhinovirus and coxsackievirus B3[31].

## Zn Can Directly Inhibit SARS-CoV-2 Replication

Antiviral properties of Zn are mainly against virus attachment, infection, and uncoating, as well as inhibition of viral protease and polymerase enzymatic processes[32]. It was stated that zinc can prevent fusion of virus with the host membrane, reduces the polymerase function, interfere with protein translation and processing, blocks release of budding virions, and destabilizes the viral envelope[33] .

At low endosomal pH Zn binds with specific histidine residue present on the viral E1 protein inhibit membrane fusion of respiratory syncytial virus, HSV, Semliki Forest virus and sindbis viruses. Finally, $Zn^{2+}$ have a potential for direct inactivation of the free Varicella-Zoster virus *in vitro*[34].

As per the research carried out by Aartjan et al. replication of various RNA viruses like influenza and polio can be damaged efficiently when intracellular $Zn^{2+}$concentration with zinc ionophore like pyrithione (PT) is increased. The combination of $Zn^{2+}$ and PT at low concentrations (2 μM $Zn^{2+}$ and 2 μM PT) inhibits the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture[35]. RdRp a core enzyme of the multiprotein replication and transcription complex (RTC) catalyzes RNA synthesis activity of these two related Nidoviruses[35]. Zn stongly inhibits the RNA-synthesizing activity of nidoviruses (including SARS-CoV) *in vitro*, which is due to change in RdRp activity during the elongation phase of RNA synthesis, probably by interfering with template binding[35].

## Synergistic Effect Of Zn With Anti-viral Drugs

Zinc supplementation can favour COVID-19 treatment when used with anti-viral drugs like ribavirin, lopinavir/ritonavir and antibiotics such as azithromycin, doxycycline.

It was suggested that zinc supplementation increases the tolerance to IFN-α-2a and ribavirin when used as complementary therapy in chronic hepatitis C patients[36].

However supplementation of Zn for 24 week had reduced the

abdominal discomfort without any additional effect on the anti hepatitis C virus dual therapy of IFN α 2 and ribavirin[37].

ZnSO4 supplementation in HIV-infected individuals was found to be effective in management of atazanavir-ritonavir-related unconjugated hyperbilirubinemia in some patients as described by Moyle G et al.[38].

### Zinc Supplementation

Zinc can be used as adjuvant therapy in a different formulation such as Zn-gluconate, Zn-acetate, Zn-sulfate, and Zn-picolinate. But the amount of elemental Zn in each salt varies. The recommended daily allowance of Zn will alter according to the sex, age and health conditions of an individual. The recommended daily allowance for healthy adults is typically 15–30 mg of elemental Zn. However long-term high-dose Zn consumption will cause a decrease of high-density lipoprotein cholesterol levels, anemia, copper deficiency, and possible genitourinary complications despite the beneficial effects of Zn in immune response[39].

## CONCLUSION

Zn possess antiviral activities by various mechanism starts from penetration to budding of new virion and also inhibits the RdRp enzyme. RdRp is the central enzyme for viral replication and transcription. Zn has demonstrated against various nidovirusfor which SARS-Cov 2 belongs. the above statements suggest that Zn prophylaxis can reduce the morbidity of affected patient.

## REFERENCES


1. Su S, Wong G, Shi W et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 2016; 24:490–502.
2. Zhu N, Zhang D, Wang W et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020; 382:727–733.
3. Forni D, Cagliani R, Clerici M et al. Molecular evolution of human coronavirus genomes. Trends Microbiol. 2017; 25:35–48.
4. Cheng VCC, Lau SKP, Woo PCY et al. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. ClinMicrobiol Rev. 2017; 20:660–694.
5. Wu A, Peng Y, Huang B et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. 2020; 27: 325–328.
6. Lu R, Zhao X, Li J et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020; 395:565–574.
7. Wrapp D, Wang N, Corbett KS et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020; 367: 1260–1263.
8. Ou X, Liu Y, Lei X et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020; 11:1620.
9. Kuba K, Imai Y, Rao S. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005; 11:875–879.
10. Liu L, Wei Q, Alvarez X et al. Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. J Virol. 2011; 85:4025–4030.
11. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods MolBiol. 2015; 1282:1–23.
12. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nat Rev Microbiol. 2009; 7:439–450.
13. Morse JS, Lalonde T, Xu S et al. Learning from the past: possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. Chembiochem. 2020; 21: 730–738.
14. Devaux CA, Rolain J-M, Colson P et al. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrob Agents. 2020; 105938:105938.
15. Wong CK, Lam CW, Wu AK et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. ClinExpImmunol. 2004; 136:95–103.
16. Rahman, M.T., Idid et al. Can Zn Be a Critical Element in COVID-19 Treatment? *Biol Trace Elem Res*. 2020
17. Catalanotto FA, Nanda R. The effects of feeding a zinc-deficient diet on taste acuity and tongue epithelium in rats. J Oral Pathol. 1977; 6:211– 20.
18. Hasan R, Rink L, Haase H. Chelation of free Zn(2)(+) impairs chemotaxis, phagocytosis, oxidative burst, degranulation, and cytokine production by neutrophil granulocytes. Biol Trace Elem Res. 2016; 171:79–88.
19. Lian H, Zang R, Wei J el al. The zincfinger protein ZCCHC3 binds RNA and facilitates viral RNA sensing and activation of the RIG-I-like receptors. Immunity. 2018; 49:438–48.e5.
20. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006; 124:783–801.
21. Lei CQ, Zhong B, Zhang Y et al. Glycogen synthase kinase 3β regulates IRF3 transcription factor-mediated antiviral response via activation of the kinase TBK1. Immunity. 2010; 33:878– 89.
22. Mao AP, Li S, Zhong B et al. Virus-triggered ubiquitination of TRAF3/6 by cIAP1/2 is essential for induction of interferon-beta (IFN-beta) and cellular antiviral response. J Biol Chem. 2010; 285:9470– 6.
23. Berg K, Bolt G, Andersen H et al. Zinc potentiates the antiviral action of human IFN-alpha tenfold. J Interferon Cytokine Res. 2001; 21:471– 4.
24. Haase H, Rink L. Functional significance of zinc-related signaling pathways in immune cells. Annu Rev Nutr. 2009; 29:133–52.
25. von Bülow V, Dubben S, Engelhardt G et al. Zinc-dependent suppression of TNF-alpha production is mediated by protein kinase A-induced inhibition of Raf-1, I kappa B kinase beta, and NF-kappa B. J Immunol. 2007; 179:4180–6.
26. Prasad AS, Bao B, Beck FW et al. Antioxidant effect of zinc in humans. Free RadicBiol Med. 2004; 37:1182– 90.
27. Uchide N, Ohyama K, Bessho T et al. Effect of antioxidants on apoptosis induced by influenza virus infection: inhibition of viral gene replication and transcription with pyrrolidinedithiocarbamate. Antivir Res. 2002; 56:207–217.
28. Suara RO, Crowe JEJ. Effect of zinc salts on respiratory syncytial virus replication. Antimicrob Agents Chemother. 2004; 48: 783–790.
29. Korant BD, Kauer JC, Butterworth BE. Zinc ions inhibit replication of rhinoviruses. Nature. 1974; 248:588–590.
30. Lanke K, Krenn BM, Melchers WJG et al. PDTC inhibits picornavirus polyprotein processing and RNA replication by transporting zinc ions into cells. J Gen Virol. 2007; 88:1206–1217.
31. Krenn BM, Gaudernak E, Holzer B et al. Antiviral activity of the zinc ionophorespyrithione and hinokitiol against picornavirus infections. J Virol. 2009; 83:58–64.
32. Overbeck S., Rink L., Haase H. Modulating the immune response by oral zinc supplementation: a single approach for multiple diseases. Arch Immunol Ther Exp (Warsz). 2008; 56:15–30.
33. Scott A Read, Stephanie Obeid, Chantelle Ahlenstiel et al. The Role of Zinc in Antiviral Immunity. *Advances in Nutrition*. 2019 Jul 1; 10(4):696-710.
34. Shishkov S., Varadinova T., Bontchev P. et al. Complexes of zinc with picolinic and aspartic acids inactivate free varicella-zoster virions. Met Based Drugs. 1996; 3:11–14.
35. teVelthuis AJ, van den Worm SH, Sims AC et al. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity *in vitro* and zinc ionophores block the replication of these viruses in cell culture. PLoSPathog. 2010; 6:e1001176. 10.1371
36. Ko W-S, Guo C-H, Hsu G-SW et al. The effect of zinc supplementation on the treatment of chronic hepatitis C patients with interferon and ribavirin. ClinBiochem. 2005; 38:614–620.
37. Suzuki H, Takagi H, Sohara N et al. Triple therapy of interferon and ribavirin with zinc supplementation for patients with chronic hepatitis C: a randomized controlled clinical trial. World J Gastroenterol. 2006; 12:1265–1269.
38. Moyle G, Else L, Jackson A et al. Coadministration of atazanavir-ritonavir and zinc sulfate: impact on hyperbilirubinemia and pharmacokinetics. Antimicrob Agents Chemother. 2013; 57:3640–3644.
39. Saper RB, Rash R. Zinc: an essential micronutrient. Am Fam Physician. 2009; 79:768–772.