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Review Paper

Bacteriolyses by Zn^{2+}-induced PGN autolysins, and virucides by Zn^{2+} released from ZAP, ZnONPs against 2019-nCoV prevention, respiratory and pulmonary COVID-19

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Abstract

Bacteriolyses of bacterial cell walls by Zn^{2+}-induced peptidoglycan autolysins, and virucides by zinc-finger antiviral protein (ZAP), zinc binding domain (ZBD), zinc oxide nanoparticles (ZnONPs) against human coronavirus and 2019-nCoV or COVID-19 infection are discussed respectively. Bacterial peptidoglycan (PGN) autolysin AmiA for *S. aureus* amidase is acted on PGN binding and cleavage. The AmiA distinguishes PGN mostly by the peptide, and the cleavage is facilitated by a zinc-activated water molecule, in order to develop new therapeutics against *MRSA*. Lytic amidase autolysin LytA associates with the cell wall via its zinc-binding motif. Autolysin LytF is endopeptidase in *B. subtilis* that plays a role in cell separation and hydrolase of the peptide. Thus, Zn^{2+} ions induced PGN autolysins for *S. aureus* is amidase LytA and endopeptidase LytM that are anticipated to be used as antibacterial potential of endogenous PGN-degrading enzymes against *S. aureus*. Zinc-dependent endopeptidases (Eps) are predicted to hydrolyze PGN to facilitate cell growth that zinc availability affects strong activity of cell wall hydrolases, and zur-regulated endopeptidases are present in divergent Gram-negative bacteria. AmiB catalyzes the degradation of PGN in Gram-negative bacteria, resulting in a marked increases of sensitivity to oxidative stress and organic acids. Eps at outer membrane lipoprotein and amidase, peptidase, and carboxy-peptidase in PGN layer against *E. coli* are anticipated to be employed as *E. coli* cell wall-hydrolyzing enzymes of anti-bacterial potential. Zinc-dependent PGN autolysin of amidases are enhanced the anti-bacterial activities against both Gram-positive and Gram-negative bacteria. The autolysin-mediated bacteriolysis induced bacterial cell death can contribute to the bactericidal activities. On the other hand, enveloped viruses enter cells and initiate disease-causing cycles of replication in virus-cell interaction. ZNF ZCCHC3 binds RNA and facilitates viral RNA that is critical for RIG-1 like receptor (RLR)-mediated innate immune response to RNA virus. ZAP inhibits entry, replication, and spread of certain viruses, and promotes viral RNA degradation. ZAP also may regulate DNA and RNA virus replication that ZAP controls Retroviral RNA production and HIV-1 infection by promoting the degradation of specific viral mRNAs. ZBD inhibits Nidovirus RNA synthesis and replication, hence the 2019-nCoV may be regulated by ZBD. ZnONPs recently are used in various applications of veterinary science due to their antibacterial and antiviral agents, tissue repair that the ZnONPs are anticipated to be employed in prevention of human coronavirus infection. 2019-nCoV is RNA virus that has high mutation rate, and these high rates are correlated with enhanced virulence and evolvability, traits considered beneficial for viruses. 2019-nCoV (β-CoV) structure has a spike glycoprotein (S) that the coronavirus protein mediates coronavirus entry into host cells which the evolution of these two critical functions of coronavirus spike proteins, receptor recognition and membrane fusion must be considered to be able to degrade or suppress for the spikes and the membrane by Zn^{2+}-centered tetrahed-rally coordinated binding. Zinc ions could inhibit virus entry and membrane fusion of S1 and S2 domains of spike protein with zinc ion-binding interaction. Zn^{2+}

ions can prevent in the early stage of 2019-nCoV infected patient with antiviral zinc homeostatic immunity and have important roles for respiratory and pulmonary process of COVID-19 disease. The antiviral compounds including zinc N-ethyl-N-phenyldithiocarbamate (EPDTC) inhibit the viral protease, preventing humancoxsackie virus strain B3 (CVB3) genome replication. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C and 3C-like proteases. Thus, Zinc ion drug development is anticipated to be adopted by using ZAP viral gradation via cell surface receptors and Zn^{2+}-coordination pattern, causing to lead enhancement of the anti-viral activity.

Keywords: Bacterial PGN autolysin, Autolysin amidase, ZAP/ZBD/ZnONPs, 2019-nCoV RNA mutation, Preventative, respiratory and pneumonic COVID-19, Zn^{2+}-coordination pattern.

Abbreviations: **Aas=**autolysin/adhesin of *Staphylococcus saprophyticus,* ***ABC=***ATP-binding cassette, **APC=**antigen presenting cell, **ARDS=**acute respiratory distress syndrome, ***A. stephensi=****Anopheles stephensi,* ***B. abortus=*** *Brucella abortus,* ***B. subtilis=****Bacillus subtilis,* **CBDs=**cell wall binding domains, **CBPs=**choline binding proteins, ***C. difficile=*** *Clostridium difficile,* **CKD=**Chronic Kidney Disease, **CMV=**Cucumber mosaic virus, **CVB3=**humancoxsackievirus strain B3, ***E. coli=****Escherichia coli,* ***E. faecalis=*** *Enterococcus faecalis,* **EPDTC=**N-ethyl-N-phenyldithiocarbamate, **ETEC=**Enterotoxigenic *E. coli,* ***Eps=****Zinc dependent endopepti-dases,* ***FnBPs=*** fibronectin-binding proteins, **Gas=**group A streptococcus, **GelE=**gelatinase, **HCoV=**human coronavirus, **HCV=** hepatitis C virus, **HD=**hemodialysis, **HIV-1=**Human immunodeficiency virus type 1, **IFITMs=**Interferon transmembrane proteins, ***M. catarrhalis=****Moraxella catarrhalis,* **MCPs=** Metallocarboxy-peptidases, **MIBRs=** most probable immunoprotective B-cell epitope regions, **MRB=** multidrug bacteria, **ORSs=**oral rehydration solutions, **ORT=**oral rehydration therapy, ***P. aeruginosa=*** *Pseudomonas aeruginosa,* **PBP2a=**penicilline-binding protein2a, **PGN=**peptidoglycan, **PGRPs=**peptidoglycan recognition proteins, **PSP=** plasmid stabilization protein, **RdRp= RNA-dependent RNA polymerase, RIG-1=**retinoic acid-inducible gene-1, **RLR=**RIG-1 like receptor, **ROS=**reactive oxygen species, **Sags**= super-antigens, **SasG**=*S. aureus* surface protein, ***S. aureus=****Staphylococcus aureus,* **SBP**= solute-binding protein, **SEB**=staphylococcal entoxin serotype B, **SINV=**Sindbis virus, **SOD=**superoxide dismutase, ***S. pneumoniae=*** *Streptococcus pneumoniae,* **SSP=**stable signal peptide, **TBVs=** transmission-blocking vaccines, **Tsip1=**Tsip1-interac-ting protein 1, **VRE=**vancomycin-resistant *Enterococcus faecium*, **ZAP=**zinc-finger antviral protein, **ZBD=**zinc-binding domain, **ZBL=**zinc binding lipoprotein, **ZNF^{EB}**=EBV-induced zinc finger gene, **ZnONPs=**zinc oxide nanoparticles, **ZnuA=**Zinc uptake A.

Introduction

Zinc is a nutritionally fundamental trace element in human body that zinc homeostasis is a key factor in maintaining a healthy immune system. Average values of recommended intake may be 7~11mg/day for adults. Zinc deficiency currently accounts for approximately 16% of lower respiratory tract infections, 18% of malaria, and 10% of diarrheal diseases, while severe zinc deficiency is rare, mild to moderate deficiency is more common worldwide[1]. Zinc excess provokes an impairment of the immune system and has significant toxicity to bacteria. Zinc is known to be essential for highly growth and development of all organisms in the human body, especially the immune system. A variety of effects of zinc on immune cells depend on the zinc concentration that in a concent-ration of 100 µmol/L, zinc suppresses natural killer cell killing and T-cell function whereas monocytes are activated direcly, and in a concentration of 500 µmol/L, zinc evokes a direct chemotactic activation of neutrophil granulocytes[2]. The zinc ions play important roles of effects on prevention and reduction for bacterial and viral infections. Bacteria and viruses are most common cause of foodborne disease by food poisoning and many human illnesses are caused by bacterial and viral infections. Bacterial and viral infections are associated with deficiencies in macronutrients and micronutrients, including the essential trace elements that dietary supplementation to provide adequate element supply has been proposed to confer health benefits for patients suffering from some bacterial or viral diseases[3]. The treatments with herbal nutraceuticals and zinc likely indirectly contributed to the increase in the resistance of the lambs against *Haemonchus contortus* infection[4]. Zinc is the second most abundant trace metal with human body 2~3g and a plasma concentration of 12-16 µM, 90% in muscle and bone, and 10% other organs include prostate, liver, the gastrointestinal tract, kidney, skin, lung brain, heart, and pancreas in humans that cellular zinc underlies an efficient homeostatic control that avoids accumulation of zinc in excess. Host zinc homeostasis changes in response to bacterial infections,

including production of metal sequestering proteins and bombardment of bacteria with toxic level of zinc at host-pathogen interface[5].

Apoptosis is defined as cell death activated by an internally controlled suicide program that bacteria are able to trigger apoptosis, including the secretion of compounds such as protein synthesis inhibitions, pore forming proteins, molecules responsible for the activation of the endogeneous death in the infected cell, and super antigens[6]. Zinc influences apoptosis by acting on several molecular regulators of programmed cell death and zinc deficiency caused by malnutrition and foods with low bio-availability, aging, certain diseases, and deregulated homeostasis is a far more common risk to human health without intoxication[7]. The influence of zinc on apoptosis is tissue/cell type, zinc concentration, and expression of zinc transporters and zinc-binding proteins.

The other, zinc deficiency in Chronic Kidney Disease (CKD) patients may be due to fecal excretion or decrease in its absorption that zinc concentrations were lower in hemodialysis (HD) patients compared to controls and Zn concentration 69.16 μg/dL of blood in HD patients, however, revealed no correlation among serum Zn concentration and anemia, serum parathyroid hormone concentration or pruritus severity in HD patients[8].

Bacterial killing of Zn^{2+} ions occurs chiefly by bacteriolyses of bacterial cell walls due to activated peptidoglycan (PGN) autolysins such as amidases, endopeptidases, and carboxypeptidase against bacteria[9]. PGN autolysins induced anti-bacterial vaccine activity may be enhanced by activation of zinc dependent PGN autolysins. PGN autolysins are bacterial peptidoglycan degrading enzymes that these muropeptides can be produced or modified by the activity of bacterial glycolytic and peptidolytic enzymes referred to as PGN hydrolases and autolysins which specific bacterial pathogens use PGN degradation to subvert host innate immunity[10]. Bact-eria have to avoid recognition by the host immune system in order to establish a successful infection which bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system[11].

Viruses are intracellular obligate parasites that cause infection by invading cells of the body. Their life cycle comprises a short extracellular period and a longer intracellular period during which they undergo replication. The immune system has non-specific and specific mechanism that attacks the virus in both phases of its life cycle which specific antibodies protect against viral infections and play an important role in antiviral immunity, mainly during the early stage of the infection[12]. To date, there are presence as severe problems in the world against the infection of new-typed human coronavirus (HCoV) of 2019-novelCoV. The rapid final completion is anticipated.

In this review article, firstly, bacteriolyses of bacterial cell walls by Zn^{2+} ions induced autolytic PGN activation are debated against *Staphy-lococcus aureus* (*S. aureus*) as Gram-positive bacterium and *Escherichia coli (E. coli)* as Gram-negative bacterium. Secondly, virucidal activities of Zn^{2+} released from ZAP, ZnONPs are discussed against 2019-nCoV prevention, respiratory and pulmonary COVID-19. Lastly, the bacteriolytic and the virucidal mechanisms are clarified respectively.

Zinc-induced bacterial and viral immunity to maintain constant homeostasis

Zinc-induced anti-bacterial immunity is important factor to be both essential and toxic for microorganism that zinc(II) ions have crucial roles in many facets of the immune system, in which microbial susceptibility to Zn(II) toxicity is mediated by extracellular cation competition and this susceptibility can be harnessed by innate immune response[13]. It is essential for bacteria to maintain metal ion homeostasis that the need for tight homeostatic control is particularly true for zinc, an essential transition metal ion which zinc ions may be used as an antimicrobial agent in the innate immune system and zinc efflux is an important contributor to group A *Streptococcus* (GAS) pathogenesis[14]. Maintaining a constant state of cellular zinc homeostasis is essential for normal function that typically, human zinc intakes range from 107 to 231 μmol/dm^3, this is equivalent to 14∼30 mg/kg for comparison with rat diets[15]. Zinc-modulating immune response depends on a sufficient availability of this zinc that zinc supplementation in diseases diarrhera, chronic hepatitis pneumonia, and acute lower respiratory infection seems beneficial[16]. Zinc induced antiviral immunity plays an important role in antiviral antibodies that zinc binds to the viral envelope or capsid proteins, and block the virus from entering into host cell, in which cytotoxic T lymphocytes (CTL) cells in specific antiviral immunity recognize viral antigens presented at the cell surface associated with I major histocompatibility complex (MHC) molecule[17]. An essential trace element zinc is crucial for growth, development, and the maintenance of immune function which zinc status is a critical factor that can influence antiviral immunity, particularly as zinc-deficient populations are often most at risk of

acquiring viral infections such as HIV, HCV[18]. Common features possess that enveloped viruses enter cells by membrane-fusion protein on the surface, fusion glycoprotein on metastable prefusion and interactions with neutralizing antibodies. Implications for immunogen design of next-generation vaccines have been shown from the results that stable immunogens presenting the same antigenetic sites as the labile wild-type proteins efficiently elicit potently neutralizing antibodies[19].

Bacteriolyses by Zn^{2+} ion-induced PGN autolysin activation

Molecular structures of *S. aureus* and *E. coli* cell walls and the action sites of PGN autolysins
Bacterial PGN structure of both Gram-positive and Gram-negative bacteria comprises repeating disaccharide backbones of N-acetylglucosamine (NAG) and β-(1-4)-N-acetylmuramic acid (NAM) that are crosslinked by peptide stem chains attached to the NAM residues[20]. The action sites of bacterial autolysins are comprised that for *Staphylococcus aureus* (*S. aureus)* PGN layer cell wall, there are N-acetylmuramidase-L-alanine amidase and PGN chain cross-linkage DD-endopeptidase.

The other, for *Escherichia coli* (*E. coli)* cell wall**,** there are endopeptidase of degrading enzyme at lipoprotein of C- and N-terminals, and amidase, peptidase, and caboxypeptidase at thin PGN layer in periplasmic space[21]. The bacterial cell walls are a strong flexible mesh work of PGN that gives a bacterium structural integrity, in which to accommodate a growing cell, the walls are remodelled by PGN synthesis and PGN autolysin. PGN is the main constituent of bacterial cell walls and must be continuously synthesized and degraded to maintain the integrity and viability of the cells that bacterial cell wall hydrodases of amidase, gycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosy-lases as well as cell wall binding domains[22]. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial activities.

Zn^{2+} ions induced activated PGN autolysins promote the bacteriolysis against *Gram-positive* bacterial cell wall
S. aureus amidase AmiA is acted on PGN binding and cleavage. The AmiA distinguishes PGN mostly by the peptide, and the cleavage is facilitated by a zinc-activated water molecule, in order to develop new therapeutics against *MRSA*[23]. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of *Staphylococcus saprophyticus*) is inhibited and is necessary for the C-terminal GW repeats, not the N-terminal repeats[24]. Lytic amidase autolysin LytA which is released by bacterial lysis, associates with the cell wall via its zinc-binding motif that the amidase domain comprises a complex substrate-binding crevice and needs to interact with a large-motif epitope of PGN for catalysis[25]. Suicidal amidase autolysin LytA having both autolysis and capsule shedding depends on the cell wall hydrolytic activity of LytA that capsule shedding drastically increases invasion of epithelial cells and is the main pathway by which pneumococci reduce surface bound capsule during early acute lung infection of mice[26]. The LytB PGN hydrolase responsible for physical separation of daughter cells cleaves the GlcNAc-β-(1,4)-MurNAc glycosidic bond of PGN building units that cell wall digestion products and solubilisation rates might indicate a tight control of LytB activity to prevent unrestrained breakdown of the cell wall[27]. The PGN-remodeling autolysins LytC, LytD, and LytF are expressed in the same subpopulation of cells and complete flagellar synthesis that LytC appears to be important for flagellar function, motility was restored to a LytC mutant by mutation of either lon A, and LytC, LytD,and LytF autolysins to population heterogeneity in *B. subtilis*[28].

Atl is the major autolysin in *S aureus* that the bifunctional major autolys in play a key role in staphylococcal cell separation which processing of Atl yield catalytically active amidase and glucosamidase domains[29]. The biochemical and strucural staphylococcal Atl have successful cloaning, high level over-expression, and purification Atl proteins[30]. AtlA is the major PGN hydrolases of *Enterrococcus faecalis* involved in cell division and cellular autolysis and the zinc metalloprotease, gelatinase (GelE) of their interplay proposed to regulate AtlA function, which N-terminal cleavage was required for efficient AtlA-mediated cell division, and AtlA septum localization and subsequent cell separation can be modulated by a single GelE-mediated N-terminal cleavage event[31]. Major Atl autolysin also have an essential role in the early events of the fibronectin-binding proteins (FnBPs)-dependent *S. aureus* biofilm phenotype[32]. For the contribution of autolysins of PGN hydrolases to bacterial killing, there are N-acetyl-glucosaminidase (AtlA), two N-acetyl-muraminases (AtlB and AtlC)[33].

Endopeptidase of autolysin LytF in *B. subtilis* plays a role in cell separation and hydrolase of the peptide[34]. Endopeptidase of autolysin LytM is a glycyl-glycyl endopeptidase, hydrolyzing the pentaglycine interpeptide crossbridge[35]. Thus, Zn^{2+} ions induced PGN autolysins for *S. aureus* is amidase LytA and endopeptidase LytM that are anticipated to be used as antibacterial potential of endogenous PGN-degrading enzymes against *S. aureus*[36].

Zn^{2+} ions induced degrading enzyme of outer membrane lipoprotein and PGN autolysins promote the bacteriolysis against *Gram-negative* bacterial cell wall

Zinc-dependent endopeptidases (Eps) are predicted to hydrolyze PGN to facilitate cell growth that zinc avaliability affects strong activity of cell wall hydrolases, and zur-regulated endopeptidases are present in divergent Gram-negative bacteria[37]. Amidase gene (AmiB) catalyzes the degradation of PGN in Gram-negative bacteria that the amiB gene was composed of 1,722 nucleotides and 573 amino acid which is involved in the separation of daughter cells after cell devision and inactivation of the amiB gene, resulting in a marked increases of sensitivity to oxidative stress and organic acids[38]. Amidase activity of amiC controls cell separation and PGN fragments release[39]. Zinc-regulated peptidase maintains cell wall integrity during immune-mediated nutrient sequestration against *Acinetobacter baumannii*[40].

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides that the carboxypeptidase B1 of and its evaluation have been high molecular characterization for transmission-blocking vaccines (TBVs) against Malaria eradication[41]. Metallo-carboxypeptidases (MCPs) of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei or cruzi*[42]. Zinc-depedent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial activities. Thus, Endopeptidase at outer membrane lipoprotein and Amidase, peptidase, and carboxypeptidase in PGN layer against *E. coli* are anticipated to be employed as *E. coli* cell wall-hydrolyzing enzymes of antibacterial potential.

Human peptidoglycan recognition proteins (PGRPs) are novel class of recognition and effect or molecules with broad Zn^{2+}-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria[43]. Hence, zinc-dependent PGN autolysin of amidases are enhanced the anti-bacterial activities against both Gram-positive and Gram-negative bacteria. Thus, autolysin-mediated bacteriolysis induced bacterial cell death can contribute to the bactericidal activities. Further, autolysin-mediated lysis-induced bacterial cell death may make contribution to the bacteriolytic vaccine activities.

Accordingly, anti-bacterial activities of bacteriolyses by Zn^{2+} ions induced activated PGN autolysins against *Gram-positive* thick PGN layer, and Gram-negative outer membrane lipoprotein and thin PGN layer cell walls are represented in Table 1.

Table 1: Zinc induced bacteriolysis against Gram-positive thick PGN envelope cell wall and Gram-negative lipoprotein and thin PGN layer cell wall

Zn^{2+} Ions	**Gram-Positive PGN Layer Cell Wall**
Zn^{2+}	**→ Zn^{2+}, O_2^-, H_2O_2, ·OH, ·NO, $ONOO^-$** Zn^{2+} ions induced activated PGN autolysins ·*S. aureus* amidase AmiA ·Recombinant amidase of the Aas ·Lytic amidase LytA for *Streptococcus pneumoniae* ·*Pneumococcal autolysin* LytA LytC, D, F of PGN remodeling for Bacillus subtilis ·Endopeptidase LytF for bacillus subtilis ·AtlA autolysin for GelE against *E. faecalis* ·AtlA, AtlB, AtlC autolysins against *enterococcus faecalis* ·Fusion protein autolysin, MIBRs against *S. pneumoniae* ·Metallocarboxy peptidase M32 against *Trypanosoma brucei* or cruzi ·PBP2a and autolysin mixture aginst MRSA
Zn^{2+} ions	**Gram-Negative Cell Wall**
	Outer Membrane Lipoprotein at C- and N-terminals / **Periplasmic Space Thin PGN Layer**
Zn^{2+}	**→ Zn^{2+}, O_2^-, H_2O_2** / **→ Zn^{2+}, O_2^-, H_2O_2, OH^-, · OH**
	·Amidase gene amiB/LysM · Endopeptidase regulation of ShyA and ShyB · Outer membrane receptor against *N. menigitidis* · ETEC subunit vaccine · ZnuB against *P. aeruginosa.* · Preventive vaccine by recombinant flagella *against P. aeruginosa* / · AmiC in PGN fragment release · Carboxypeptidase by transmission-blocking vaccines ·PGRPs have Zn^{2+}-depedent bactericidal activity · D-glutamate auxotrophy against *P. aeruginosa* PA14 · Zn in infectious *diarrhoea* · *ZnuA against P. aeruginosa* · Recombinant flagella and pili against *P. aeruginosa* ·Carboxypeptidase B1 against *Anopheles stephensi* and for malaria as transmission- blocking

Virucidal activities of zinc-finger antiviral protein, zinc-binding domain, and zinc oxide nanoparticles against viral infection

Zinc-finger protein

Zinc salts (Zn^{2+} concentration: < 80 μM) suppressing the activity of viral RNA-dependent RNA polymerase, brock hepatitis E virus (HEV) replication[44]. Respiratory syncyyial vitrus (RSV) is the most important viral cause of acute respiratory tract infection (ARI) that the complete inhibitory effect of zinc salts (Zn^{2+} concentration: 1 and 10 mM) on RSV plaque formation was observed, in which zinc(II) ions mediate antiviral activity on RSV by altering the ability of the cell to support RSV replication[45]. The designed polydactyl zinc-finger protein (ZNF) is prepared consiting HIV-1 type integrase fused to the synthetic zinc finger protein E2C that offers an efficient approach and a versatile framework for directing the integration of retroviral DNA into a predetermined DNA site[46]. The ZNF ZCCHC3 binds RNA and facilitates viral RNA that ZCCHC3 is a co-receptor for the retinoic acid-inducible gene-1 (RIG-1) and antigen MDA5 which is critical for RIG-1 like receptor (RLR)-mediated innate immune response to RNA virus[47]. Artificial ZFN were targeted to the high affinity Sp1-binding site, and by being fused with TatdMt and POZ domain, they strongly block both Sp1-cyclin T1-dependent transcription and Tat-dependent transcription of HIV-1 [48].

Zinc-finger antiviral protein

The zinc-finger antiviral protein (ZAP) controls virus entry, DNA/RNA replication, and spreading against viral infection. The ZAP in first steps of HCV infection may be used as entry inhibitor[49]. Interferon induced transmembrane proteins (IFITMs) inhibit the cellular entry of a broad range of viruses that IFITM-mediated restriction requires recognition of viral RNA elements[50]. The interferon-stimulated genes serve as enhancers of antiviral innate immunity[51]. ZAP inhibits alphavirus replication that elucidation of the antiviral mechanism by which ZAP inhibits Sindbis virus (SINV) translation may lead to the development of agents with broad activity against alphaviruses[52]. The ZAP also inhibits Influenza A virus (IAV) protein expression, in which suggests an important role of ZAP in the host effort to control IAV infection and the importance of the threat of ZAP to the virus[53]. The host cell restriction factors that limit IAV have been investigated[54]. ZAP may regulate DNA and RNA virus replication. Inhibition of bacterial DNA replication during nitrosative stress is accompanied by zinc mobilization[55]. ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation[56]. ZAP inhibits Retroviral RNA production[57] and ZAP inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs[58].

ZAP regulates spread

ZAP' stress with antiviral activity and induced virus replication are regulated upon virus infection to inhibit virus spread[59]. ZAP-70 kinase regulates HIV cell-to-cell spread that HIV usurps components of the immunnological synapse machinery to ensure its own spread through cell-to-cell contacts[60]. An understanding of viral cell-to-cell transmission spreading will enhance our ability to intervene in the efficient spreading of viral infection[61].

Zinc-binding domain

A novel zinc-binding domain (ZBD) is essential for formation of the functional Junin virus envelope glycoprotein complex that the envelope glycoprotein of the Junin arenavirus (GP-C) mediates entry into target cells through a pH-dependent membrane fusion mechanism, in which this unusual motif may act to retain a cleaved 58-amino-acid stable signal peptide (SSP) for its role in modulating membrane fusion activity[62]. Entry of the virus into the host cell is mediated by the viral envelope glycoprotein, GPC that SSP was retained in GPC through interaction with a zinc-binding domain (ZBD) in the cytoplasmic tail of transmembrane fusion of G2 subunits that Junin virus ZBD displays a novel fold containing two zinc ions, in which the structural basis for retention of the unique SSP submit suggests a mechanism whereby SSP is positioned in the GPC complex to modulate pH-dependent membrane fusion[63]. In addition, ZBD inhibits Nidovirus RNA synthesis and replication[64], hence the 2019-nCoV may be regulated by ZBD**.**

Viral membrane fusion protein

Enveloped viruses enter cells and initiate disease-causing cycles of replication that in all cases virus-cell fusion is executed by one or more viral surface glycoproteins denoted as the fusion protein, in which the structure and mechanisms on viral membrane fusion protein are important problems[65]. The membrane fusion reaction, membrane interaction, conformational changes of specialized virus envelope proteins, and refolding reactions of specific fusion proteins can mediate both virus-cell fusion leading to infection and pathological cell-cell fusion, in which they are increasingly viewed as targets for antiviral intervention[65].

Zinc oxide and ZnONPs inhibit virus entry, replication, and spread

Zinc oxide tetrapods (ZnOTs) of micro-nanostructures block HSV-2 attachment, entry into host cell, and stop the spread of the virus among already infected cells[66]. ZnOTs can inhibit HSV-1 growth and spread in corneal tissues, hence, the ZnOTs also could control HCoVs[67]. Zinc has broad-spectrum antiviral activity against HIV, transmissible gastroenteritis virus (TGEV), and SARS-CoV that 2,500 mg/kg diet Zn^{high} showed a down-regulation of interferon (IFN)-α, oligoadenylate synthetase (OAS), Zn transporter (ZIP4), as well as the Zn transporters (ZnT1) and (ZnT5), in which the Zn^{high} documented an earlier and higher sysmatic TGEV-specific serum antibody response[68].

Zinc oxide nanoparticles (ZnONPs) recently are used in various applications of veterinary science due to their antibacterial and antiviral agents, tissue repair that the ZnONPs are anticipated to be employed in prevention of human coronavirus infection. Firstly, ZnONPs inhibit H1N1 influenza virus entry into the host cells[69]. ZnONPs-5 μmg/ml specifically regulated the correlation of microRNAs and their targeted genes[70]. Zinc oxide nanoparticles to dimethylnitrosamine (DMN) treated rats inhibit the production of mRNA of inflammatory cytokines and reduce lipid peroxidation, oxidative stress and fibrosis in the liver[71].

Zinc induced Human Coronavirus prevention for respiratory and pulmonary inflammation HCoVs infection

Coronaviruses (CoVs) and arteriviruses are related human and animal pathogens that belong to the Coronaviridae family in the order Nidovirales that CoVs have the largest known RNA genomes. The RNA-dependent RNA polymerase (RdRp) and enzymatic functions for nidoviruses that are characterized by polycistronic plus-stranded RNA genome are required to suppress the consequences of the typically high error rate of viral RdRps that the RdRp behaviour and interactions during RNA synthesis, and subsequent processing must be understandable[72]. Zinc ions are essential for the rescue of the enzymatic activities of nidovirus helicases that a complex zinc finger can inhibit possibly virion biogenesis, nidovirus replication and transcription, and dysrupting RNA synthesis[64]. Human coronaviuses (HCoVs) are recognised as coronaviruses (CoVs) associated with multiple respiratory diseases of varying severity, including common cold, pneumonia and bronchilitis that to date, seventh known HCoVs have been identified, namely HCoVs-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and recently new-typed 2019-nCoV, subsequent phylogenetic studies pointed to the bat origin of SARS-CoV based on sequences of SARS-like virus found in bats[73]. The new developed drugs for the 2019-nCoV has been now reported that Lopinavir/Ritonavir, Nucleoside analogues, Neuraminidase inhibitors, Remdesivir, peptide (EK1), abidol, RNA synthesis inhibitors (such as TDF, 3TC), anti-inflammatory drugs (such as hormones and other molecules), Chinese traditional medicine, such ShuFengJieDu Capsules and Lianhuaqingwen Capsule, could be the drug treatment options for 2019-nCoV. However, the efficacy and safety of these drugs for 2019-nCoV still need to be further confirmed by clinical experiments[74]**.** HCoVs are a well-known cause of respiratory infections, in which Zn^{2+} inhibits coronavirus and arterivirus RNA polymelase activity, and zinc ionophores block the virus replication that the combination of Zn^{2+} and pyrithione at low concentrations (Zn^{2+} concentration 2 μM) inhibits the replication of SARS-CoV and arterivirus RNA[75]. High zinc ion concentration (10 and 100 μM) to the addition of compounds and Zn concentration increasing in Cu/Zn brasses were found to inhibit the replication of various RNA virus, influenza viruses, respiratory syncytial virus and human coronavirus 229E[76].

Replication of SARS-CoV requires proteolytic processing of the replicase polyprotein by two viral cystein proteases, a chymotrypsin-like protease (3CLpro) and a papain-like protease (PLpro). This PLpro is important for development of antviral drug that would inhibit viral replication and reduce mortality associated with outbreaks of SARS-CoV that a model of PLpro in complex with ubiquitinaldehyde reveals well defined sites within the catalytic cleft that help to account for strict substrate-recognition motifs[77]. The MERS-CoV PLpro blocking loop 2 (BL2) structure differs from that of SARS-CoV PLpro, where it has been proven to play a crucial role in SARS-CoV PLpro inhibitor binding that inhibitor recognition specificity of MERS-CoV PLpro may differ from that of SARS-CoV PLpro. Inhibitory activity, of this compound was selective for SARS-CoV and MERS-CoV PLpro enzymes over two human homologues, and the ubiquitin C-terminal hydrolases[78]. The papain-like protease 1 ($PL1^{pro}$) domain is present in nonstructual protein 3 (nsp3) of alpha corona viruses and subgroup 2a beta corona viruses, and the papain-like protease 2 ($PL2^{pro}$) is present in SARS-CoV. In combination with the prior characterization of PL2pro from other alphacorona-viruses of human corona viruses 229E, NL63, these viruses employ two PL^{pro}s with overlapping specificities toward both viral and cellular substrates[79]. ZAPs also could probably inhibit the HCoVs that the ZAP could regulate RNA virus degradation of SARS-CoV's and MERS-CoV's RNA virus. Zn^{2+} ions are capable of inhibiting PLpro activity and the zinc conjugates to inhibit SARS-CoV PLpro activity that targeting PLpro with antiviral drug may have an advantage in not only inhibiting viral replication but also inhibiting the dys-regulation of signalling cascades infected cells, leading to cell death[80]. Further, zinc conjugated complexes as SARS-CoV 3C-like

protease inhibitors play important role for this Zn^{2+}-centered coordination pattern that the zinc-coordinating inhibitor is tetrahedrally coordinated to the His^{40}-Cys^{147} catalytic dyad of CVB3 $3C^{pro}$[81,82].

In zinc induced preventative antibody, a human monoclonal antibody that neutralizes SARS-CoV-2 binds a conserved epitope on the spike receptor binding domain explaining its ability to cross-neutralize SARS-CoV and SARS-CoV-2, using a mechanism that is independent of receptor binding inhibition. This antibody will be useful for development of antigen detection tests and serological assays targeting SARS-CoV-2. Neutralizing antibodies can alter the course of infection in the infected host supporting virus clearance or protect an uninfected host that is exposed to the virus. Hence, this antibody offers the potential to prevent and/or treat COVID-19, and possibly also other future emerging diseases in humans caused by viruses from the Sarbecovirus subgenus[83].

In addition to their viral receptor functions, the receptors for coronaviruses have their own physiological functions angiotensin-converting enzyme 2 (ACE2) is a zinc-dependent carboxypeptidase that cleaves one residue from the C terminus of angiotensin peptides and functions in blood pressure regulation. ACE2 also protects against severe acute lung failure, and SARS-CoV-induced downregulation of ACE2 promotes lung injury. Aminopeptidase protein (APN) is a zinc-dependent aminopeptidase that cleaves one residue from the N-terminus of many physiological peptides and plays multifunctional roles such as in pain regulation, blood pressure regulation, and tumour cell angiogenesis[84].

Zinc regulates inflammatory response that the individual preventive and protective measures drive the personal risk of getting the disease. Zinc ions inhibit the RNA-dependent RNA polymerase, which crucially replicates copies of viral RNA in the host cells. Remdesivir inhibits coronavirus with the intact proofreading, thus renders its superior antiviral efficacy. Zn status and respiratory syncytial virus (RSV) infection. Particularly, it has been demonstrated that whole blood zinc was significantly lower in children with RSV pneumonia and Zn compounds were shown to inhibit respiratory syncytial virus replication and RSV plaque formation with a more than 1,000-fold reduction at 10 µm Zn preincubation. Thus, Zn may possess protective effect as preventive and adjuvant therapy of COVID-19 through reducing inflammation, improvement of mucociliary clearance, prevention of ventilator-induced lung injury, modulation of antiviral immunity[85].

Evidence for vitamins C, D and zinc and their roles in preventing pneumonia and respiratory infections (vitamins C and D) and reinforcing immunity (zinc) appears to look particularly promising. For vitamin D a UL of 50 µg/day is advised and for zinc a UL of 25 mg/day is recommended. Supplemental daily doses of up to about 1 g, in addition to normal dietary intake, are not associated with adverse gastrointestinal effects Zinc is also involved in inflammation, elevating inflammatory responses and inducing cell- mediated immunity, and is a key component of pathogen-eliminating transduction pathways that contribute to neutrophil extracellular traps (networks which bind pathogens) formation[86].

Zinc ions-mediated antiviral activities for respiratory ailment and pneumonia against 2019-nCoV, COVID-19

Most of the coronaviruses can cause the infectious diseases in human, currently, there are four coronaviruses: α-CoV, β-CoV, γ-CoV, and δ-CoV that α-CoV and β-CoV mainly infect the respiratory, gastrointestinal and central nervous system of humans and mammals, while γ-CoV and δ-CoV mainly infect the birds, in which 2019-nCoV or SARS-CoV-2 belongs to the β-CoV according to the phylogenic analysis based on the viral genome[87]. This β-CoV structure has a spike glycoprotein (S) that the coronavirus protein mediates coronavirus entry into host cells which the evolution of these two critical function of coronavirus spike proteins, receptor recognition and membrane fusion must be considered[88]. It is responsible for binding to the receptor on the host cell as well as mediating the fusion of host and viral membrane, including with a process driven by major conformational changes of the S protein[89]. This 2019-nCoV had a similar receptor-binding domain structure to that of SARS-CoV, despite amino acid variation at some residues[90]. In addition, 2019-nCoV has a unique four amino acid insertion between S1 and S2 domains of the spike protein, which created a potential furin or TMPRSS2 cleavage site. 2019-nCoV may increase its infectivity through the receptor binding domain recombination and a cleavage site insertion[91]. Mutations and adaptation in the S and N genes could affect virus stability and pathogenicity. As more genomes are made publicly available, analysis of the genome sequence diversity across samples has revealed the highest diversity occurring in the structural genes, especially the S protein, ORF3a, and ORF8[92]**.** Genotyping SARS-CoV-2 epidemic has caused a substantial health emergency and economic stress in the world that several molecular facets of the SARS-CoV-2 pertinent to this pandemic and the discovery of genotypes linked to geographic and temporal clusters of infectious suggests that genome single nucleotide polymorphization (SNP) signatures can be used to track and monitor the epidemic**.** Thus, the major mutations are in the critical proteins, including the S protein, RNA polymerase, RNA primase, and nucleoprotein[93]. This 2019-nCoV outbreak has caused a global pandemic resulting many infected persons and deaths worldwide that the RdRp catalyzed the synthesis of

viral RNA, is a key component of coronaviral replication/transcription as a primary targeted antiviral drug[94]**.** It is unclear whether zinc ions can suppress RNA mutation and outbreak by RNA mutation.

Zinc ion drug development is anticipated to be adopted by using ZAP Viral destruction via cell surface receptors[95] and Zn^{2+}-coordination pattern[96]. Zinc ions may be functioned with the viral spike(S), envelope(E), necleocapsid(N) proteins, and membrane(M) in human coronavirus particles by Zn^{2+}-centered tetrahedrally coordinated binding to these microproteins. Zinc is efficient for the prevention of respiratory ailment and pneumonia that the N protein of HCoV induces aggregation of human elongation factor 1-alpha, inhibiting protein translation and cytokinesis by blocking filamentous-actin bundling and proliferation of several steps in T-lymphocyte generation was significantly inhibited by the infection of recombinant retrovirus expressing HCoV N protein, in which there will result in zinc ions-induced prevention of infectious and pneumonic spreading[97]. Accordingly, appreciable Zn^{2+} ion solutions may be most effective for the prevention of coronavirus infection, subsquent respiratory illness that the lower Zn^{2+} ion concentration (< 80 μM) could be efficient for vaccine candidates and the higher Zn^{2+} ion concentration (≒100 μM) should be available for the pulmonary disease and the infectious spreading.

Clinical features associated with patients infected with SARS-CoV, MERS-CoV and SARS-CoV-2 range from mild respiratory illness to severe acute respiratory disease. Both MERS and SARS patients in later stages develop respiratory distress and renal failure Pneumonia appears to be the most frequent manifestation of SARS-CoV-2 infection, characterized primarily by fever, cough, dyspnea, and bilateral infiltrates on chest imaging that the period from infection to appearance of symptoms varies. Generally, it is thought to be days, however, a research group at Guangzhou Medical University reported the incubation period to be 24 days. In a family cluster of infections, the onset of fever and respiratory symptoms occurred approximately three to six days after presumptive exposure[98].

SARS-CoV-2 enters the target cells through the angiotensin-converting enzyme 2 (ACE2) receptor and the transmembrane protease, serine 2 (TMPRSS2). The TMPRSS2 inhibitors block the cellular entry of the SARS-CoV-II virus through the downregu-lated priming of the SARS-CoV-II spike protein. Zinc has antiviral effects, it improves immune responses and suppresses viral replication. Therefore, the 50 mg zinc per day may provide a protective role against the COVID-19 pandemic, likely by improving the host's resistance against viral infection[99,100]. Zn^{2+} inhibits coronavirus and anterivirus RNA polymelase activity, and zinc ionophores block the virus replication that the combination of Zn^{2+} and pyrithione at low concentrations inhibits the replication of SARS-CoV and arterivirus RNA. The other, high zinc ion concentration and the addition of compounds that stimulate cellular zinc ions were found to inhibit the replication of various RNA virus, influenza viruses, respiratory syncytial virus and coronaviruses[101].

In order to prevent that an outbreak of respiratory sickness caused by a novel coronavirus (Covid-19) has become a serious public threat and disrupted many lives, assessing the efficacy of FDA-approved Zn-ejector drugs such as disulfiram combined with interferon to treat Covid-19 infected patients has been proposed. Based on evolutionary and physical principles of the key factors controlling the reactivity of Zn-bound Cys, having identified putative labile Zn-sites in Covid-19 that can be targeted by Zn-ejector drugs, leading to Zn^{2+} release and viral structure/function disruption. It presents an avenue for treating Covid19-infected patients using clinically safe Zn-ejecting drugs to attack conserved catalytic and/or Zn bound cysteines in multiple targets, thus, assessing their efficacy combined with interferon in clinical settings would be of great interest. Our strategy based on evolutionary and physical principles is general and can be used to identify druggable Zn-sites in conserved domains of other viruses. Importantly, it offers a possible strategy to tackle future outbreaks of pandemic viruses: FDA-approved drugs for a certain conserved domain may be repurposed to target the same conserved domain found in a new infectious virus. Furthermore, by targeting conserved domains with druggable Zn-sites, drugs may be used to treat several types of viruses[102]. Parenteral zinc+ chloroquine/hydroxychloroquine (CQ/HCQ) with the treatment of hospitalized COVID-19 patients may help to improve clinical outcomes and to limit the COVID-19 fatality rates. Therefore, whether zinc supplementation in combination with CQ/HCQ should be recommended for high risk or also younger patients outside of clinical trials as a prevention or treatment approach during SARS-CoV-2 pandemic, should be considered only on a case-by-case basis[103,104]. SARS coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis that inflammasome-activated IL-1β levels were reduced in the lung airways of the animals infected with viruses lacking E protein ion channel activity and acute respiratory distress syndrome (ARDS) leading to death, in which E protein ion channel activity represents a new determinant for SARS-CoV virulence[105].

On the case of preventing lung and pulmonia, firstly, 2019-nCoV nucleic acid detection is carried out that accurate RNA detection of 2019-nCoV is with diagnostic value (Strong recommendation). The RNA of 2019-nCoV positive in the throat swab sampling or other respiratory tract sampling by fluorescence quantitative

polymerase chain reaction (PCR) method, especially that from multiple samples and detection kits, excluding sample quality, sample collection time, contaminatory and technical problems, is of great support for etiological diagnosis.

As antibiotic therapy, (1) Principles. Avoid blind or inappropriate use of antibacterial drugs, especially the combination of broad-spectrum antibacterial drugs. Enhancement of bacteriological surveillance should be performed and promptly given appropriate antibacterial drugs when it occurs secondary bacterial infection. (2)According to the clinical manifestations of patients, if the accompanying bacterial infection cannot be ruled out, mild patients can take antibacterial drugs against community-acquired pneumonia, such as amoxicillin, azithromycin, or fluoroquinolones, empirical antibacterial treatment in severe patients should cover all possible pathogens, deescalating therapy until the pathogenic bacteria are clarified[106].

Drug treatment,(1)At present, there is no evidence from randomized controlled trial (RCT) to support specific drug treatment against the new coronavirus in suspected or confirmed cases. (2)The α-interferon atomization inhalation can be considered (5 million U per time for adults in sterile injection water, twice a day) (Weak recommendation),lopinavir/ritonavir orally, 2 capsules each time, twice a day, can be also considered (Weak recommendation) [106].

The key strategies for preventing lung damages include avoiding direct lung infection, altering host-virus interactions, promoting immune responses, diluting virus concentrations in lung tissues by promoting viral migration to the rest of the body, maintaining waste removal balance, protecting heart function and renal function, avoiding other infections, reducing allergic reactions and anti-inflammatory. The first strategy is avoiding exposures that could result in widespread damages to lungs and taking post exposure mitigating measures that would reduce disease severity. The second strategy is reducing death rate and disability rate from the current levels to one tenth for infected patients by using multiple factors health optimization method. The double reduction strategies are expected to generate a series of chain reactions that favor mitigating or ending the pandemic[107].

Improve lung micro circulation to prevent damages to the lungs, Vitamins and essential nutrients for the immune system may be taken up and thus make a difference, deep breaching can improve energy metabolism by as much as 30% (for experienced, it may improve more), and avoiding exercise may save MET values by up to 70%, relaxation exercise can reduce blood circulation by 10% to 30%, avoiding a secondary infection can reduce burden on the immune system, reduce viral burden on lungs, kidneys and heart, and help maintain the waste balance in the lungs. Warn foods such as ginger, date, citrus, etc are known to improve blood circulation and energy production[108]. However, Zinc supplementation did not yield a statistically significant reduction in symptoms in children with severe pneumonia. Zinc supplements given during an acute episode are not beneficial in short-term clinical recovery from severe pneumonia in hospitalized children[109,110].

Transient zinc chelation N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) led to induction of an antiviral state that in cells via induction of heat shock proteins and activation of NF-κB and upregulation of downstream effectors which inhibit DENV replication. Interferon-stimulated genes (ISGs) are a large group of genes which have diverse effects on viral infections and mostly act at early stages of virus life-cycle. Therefore, cellular or tissue zinc homeostasis may also determine the efficiency with which pathogens replicate and disseminate in vivo. In the case of acute viral infections, strategies to transiently block zinc redistribution during viremic stages may inhibit viruses that depend on cellular zinc pools for replication. This would provide a window for the immune system to gain an upper hand and control viral infection. Zinc chelation abrogated dengue virus RNA replication and zinc chelation abrogated dengue virus RNA replication. Transient zinc chelation induces ER stress and antiviral response by activating NF-kappaB leading to induction of interferon signaling and zinc plays divergent roles in rotavirus and dengue virus infections in epithelial cells[111]. The antiviral compounds including zinc N-ethyl-N-phenyldithiocarbamate (EPDTC) inhibit the viral protease, thus preventing human coxsackie virus strain B3 (CVB3) genome replication. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C and 3C-like proteases[112].

Zinc induced ROS generation in respiratory and pulmonary COVID-19 infected cells

Zinc induced ROS generation in respiratory and pulmonary COVID-19 infected cells is that the univalent reduction of oxygen generates superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), all of which are reactive oxygen species (ROS). Superoxide has an unpaired electron, which imparts higher reactivity and renders it very unstable and short-lived. ROS are usually produced continuously in vivo under aerobic conditions. The production of ROS and its elimination by the anti-oxidant defense system in cells is a highly modulated process for maintaining normal physiological function in the body, in which the

nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are a group of plasma membrane-associated enzymes which catalyze the production of superoxide •O2− from oxygen by using NADPH as the electron donor[113].

In respiratory COVID-19 infected cells is that Respiratory viruses are known to induce ROS-generating enzymes, including nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases, Nox) and xanthine oxidase (XO) and to disturb antioxidant defences. Increased activities of the Nox and Dual oxidase (Duox) family were observed both in vitro and in vivo. Treatment of infected cell cultures and laboratory animals (mice) with the pan-Nox inhibitor dibenziodolium chloride (DPI) profoundly attenuated ROS production induced by IV, HRSV, and HRV. Although DPI has low specificity for Nox in comparison to other flavoproteins, a detailed analysis with other approaches revealed that several NADPH oxidases are implicated in generation of ROS. Respiratory viruses cause millions of cases of severe illness and thousands deaths each year. So far, no efficient measures for prevention and treatment exist, in which changes in redox homeostasis in infected cells are one of the key events that is linked to infection with respiratory viruses and linked to inflammation and subsequent tissue damage[114].

Zinc induced ROS generation in pulmonary COVID-19 infected cells is that Bacteria, viruses, and environmental agents account for the vast majority of episodes of exacerbation. Exacerbation, systemic inflammation, ROS generation, alterations of metabolism, cardiovascular events, and lung cancer contribute to the overall disease severity and untimely death. Such inflammatory processes, especially sustained chronic conditions of inflammation, along with inflammation-induced oxidative stress from dead or injured cells, could lead to irreversible cellular or tissue damage with the passage of time, which further contributes to the development of chronic degenerative diseases[115].

As mentioned above, Zn^{2+} ions induced antiviral activities for prevention, respiratory ailment and pulmonary disease against COVID-19 infection are represented in Table 2. However, COVID-19 degradation or destruction by zinc-finger antiviral proteins remains yet unclear and COVID-19 pulmonary care by zinc ion solutions may be of importance.

Table 2: Zn2+ ions-induced virucidal activities for prevention and antibody, respiratory ailment and pulmonary disease against COVID-19 infection

Zn^{2+} ions	**Zn^{2+} ions induced antiviral activities for prevention, and respiratory ailment and pulmonary disease against COVID-19 infection**		
	Prevention and antibody	**Respiratory infection**	**Pulmonary inflammation**
Zn^{2+}	→ Zn^{2+}	→Zn^{2+}, •O_2^-, H_2O_2, •OH	→ Zn^{2+}, •O_2^-, H_2O_2, •OH
	・Zn homeostatic immune conc 50 mg/day ・Zinc supplementation in combination with CQ/HCQ ・Zinc supplement prevents pneumonia in children ・TRPV1 prevention ・Lower Zn^{2+} conc may be efficient for vaccine candidate and higher Zn^{2+} conc may prevent respiratory ailment and acute pneumonia spreading against HCoVs	・2μM Zn^{2+} + 2μM Pyrithione (PT) inhibit RNA replication ・Higher Zn^{2+}conc + HK inhibit virus entry against DV ・Zinc chelation inhibits RNA replication ・TMPRSS2 blocks cellular entry ・FDA-approved Zn-ejector drugs such as disulfiram ・ADAR-mediated RNA editing ・2019-nCoV RNA degradation by Zn^{2+} ions? ・ZnOTs inhibit HSV-1 entry and spread ・2,500mg/kg diet ZnO has antiviral activity of SARS-CoV ・ZnONPs inhibit H1N1 influenza virus entry	・CQ/HCQ plus zinc inhibit RNA replication ・Zinc-coordinated inhibitor ・Zinc + chloroquine ・Zn-ejectors + disulfiram ・ADAR-mediated RNA editing targets ・RNA degradation by zinc ions ? ・Zinc-binding ACE2? ・ZnONPs regulate microRNA in Ovarian granulosa cells ・ZnONPs + DMN inhibit the production of mRNA of inflammatory cytokines ・ZAP degrades SARS-CoV's and MERS-CoV's RNA ・Complex zinc-finger inhibits nidovirus replication

Conclusions

Bacteriolyses by Zn^{2+} ions-induced activated PGN autolysins and virucides of Zn^{2+} ions released from ZAP, ZnONPs against 2019-nCoV prevention, respiratory and pulmonary COVID-19 infection are discussed respectively, and the bacteriolytic and virucidal mechanisms are clarified. Bacterial peptidoglycan (PGN) autolysin AmiA for Gram-positive *S. aureus* amidase is acted on PGN binding and cleavage. AmiA distinguishes PGN mostly by the peptide, and the cleavage is facilitated by a zinc-activated molecule. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of *Staphylococcus saprophyticus*) is inhibited and is necessary for the C-terminal GW repeats. Lytic amidase autolysin LytA associates with the cell wall via its zinc-binding motif. The LytB PGN hydrolase responsible for physical separation of daughter cells cleaves the GlcNAc-β-(1,4)-MurNAc glycosidic bond of PGN building units. LytC, LytD, and LytF are expressed in the same subpopulation of cells and complete flagellar synthesis.

AmiB catalyzes the degradation of PGN in Gram-negative bacteria, resulting in a marked increases of sensitivity to oxidative stress and organic acids. Amidase activity of amiC controls cell separation and PGN fragments release. Enterotoxigenic *E. coli* (ETEC) is the most common bacterial cause of children's diarrea, in which antigen and antitoxin antibodies that neutralized both toxins that are associated with all cases of ETEC diarrhea. Bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system. Autolysin mediated bacteriolysis- and zinc dependent lysis-induced bacterial cell death can contribute to the bactericidal activities, where PGN autolysins interact with biomolecules causing cell apoptosis leading to cell death. Human peptidoglycan recognition proteins (PGRPs) are novel class of recognition and effector molecules with broad Zn^{2+}-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria. In these autolysins, amidases of zinc-dependent PGN autolysin are enhanced and induced the anti-bacterial activities against bacterial infection. Thus, autolysin-mediated bacteriolysis-induced bacterial cell death can contribute to the bactericidal activities.

On the other hand, enveloped viruses enter cells and initiate disease-causing cycles of replication that in all cases virus-cell fusion is executed by one or more viral surface glycoproteins denoted as the fusion protein. ZNF is prepared consiting HIV-1 type integrase fused to the synthetic zinc finger protein E2C. ZAP specifically inhibits virus entry, replication, and spread of certain viruses, and ZAP inhibits the replication of certain viruses and promotes ZAP-mediated viral RNA degradation. Zinc oxide and ZnONPs inhibit virus entry, replication, and spread.

Human coronaviuses (HCoVs) are recognised as coronaviruses (CoVs) associated with multiple respiratory diseases of varying severity, including common cold, pneumonia and bronchilitis that to date, seventh known HCoVs have been identified, namely HCoVs-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and recently new-typed 2019-nCoV. COVID-19 may be considered to be due to RNA virus mutation that the RNA viruses have high mutation rate and these high rates are correlated with enhanced virulence and evolvability, traits considered beneficial for viruses. Zinc ions could inhibit virus entry and membrane fusion of S1 and S2 domains of spike protein with zinc ion-binding interaction. Zn^{2+} ions can prevent in the early stage of 2019-nCoV infected patient with antiviral zinc homeostatic immunity and have important roles for respiratory and pulmonary process of COVID-19 disease.

The antiviral compounds including zinc N-ethyl-N-phenyldithiocarbamate (EPDTC), were dissolved in DMSO inhibit the viral protease, thus preventing human coxsackie virus strain B3 (CVB3) genome replication. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C and 3C-like proteases**.**

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Conflicts of Interest
The author declares there is no conflict of interest.

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