



Review

Possible Benefits of Zinc supplement in CVD and COVID-19 Comorbidity



Muhammad Manjurul Karim^a, Shahnaz Sultana^b, Rokaia Sultana^b,
Mohammad Tariqur Rahman^{c,*}

^a Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh

^b Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), New Elephant Road, Dhaka 1205, Bangladesh

^c Faculty of Dentistry, University of Malaya, Kuala Lumpur 50603, Malaysia

ARTICLE INFO

Article history:

Received 1 December 2020

Received in revised form 9 September 2021

Accepted 29 September 2021

Keywords:

Angiotensinogen converting enzyme

CVD and COVID 19 Comorbidity

High blood pressure

Vasoconstriction

SARS-CoV-2

ABSTRACT

As far as comorbidity is concerned, cardiovascular diseases (CVD) appear to be accounted for the highest prevalence, severity, and fatality among COVID 19 patients. A wide array of causal links connecting CVD and COVID-19 baffle the overall prognosis as well as the efficacy of the given therapeutic interventions. At the centre of this puzzle lies ACE2 that works as a receptor for the SARS-CoV-2, and functional expression of which is also needed to minimize vasoconstriction otherwise would lead to high blood pressure. Furthermore, SARS-CoV-2 infection seems to reduce the functional expression of ACE2. Given these circumstances, it might be advisable to consider a treatment plan for COVID-19 patients with CVD in an approach that would neither aggravate the vasodeleterious arm of the renin-angiotensinogen-aldosterone system (RAAS) nor compromise the vasoprotective arm of RAAS but is effective to minimize or if possible, inhibit the viral replication. Given the immune modulatory role of Zn in both CVD and COVID-19 pathogenesis, zinc supplement to the selective treatment plan for CVD and COVID-19 comorbid conditions, to be decided by the clinicians depending on the cardiovascular conditions of the patients, might greatly improve the therapeutic outcome. Notably, ACE2 is a zinc metalloenzyme and zinc is also known to inhibit viral replication.

© 2021 The Authors. Published by Elsevier Ltd on behalf of King Saud Bin Abdulaziz University for Health Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Contents

Introduction	1687
Outline of the narrative review	1687
Pathology of CVD and angiotensinogen converting enzyme	1687
Pathology of SARS-CoV-2 infection starts with ACE-2	1687
ACE2: the epicentre of CVD and COVID-19 pathogenesis	1688
Impact of CVD treatment in COVID-19 comorbidity	1688
Alternative treatment plans for CVD and COVID-19 comorbidity	1689
How Zn ²⁺ supplement would offer a beneficial role in CVD and COVID-19 comorbidity?	1689
Conclusion	1690
Conflict of interest statement	1690
Acknowledgement	1690
References	1690

* Corresponding author.

E-mail addresses: manjur@du.ac.bd (M.M. Karim),

shahnazsultana339@gmail.com (S. Sultana), rokaiasultana@rocketmail.com

(R. Sultana), tarique@um.edu.my, m.tariqur.rahman@gmail.com (M.T. Rahman).

<https://doi.org/10.1016/j.jiph.2021.09.022>

1876-0341/© 2021 The Authors. Published by Elsevier Ltd on behalf of King Saud Bin Abdulaziz University for Health Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The 2019 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) responsible for novel coronavirus disease 2019 (COVID-19) has been declared as a pandemic on Mar 11, 2020, by World Health Organization (WHO) and driven the global health care system at bay. While the COVID-19 case fatality rate in children is nil and very low in adults up to 50 years, it is more than 20% for patients aged 80 years or more, and this was well evidenced in Europe [1].

As far as comorbidity is concerned, cardiovascular diseases (CVD) appear to be accounted for the highest prevalence, severity, and fatality among COVID-19 patients. Within the spectrum of CVD, its coexistence has been reported highest for hypertension (35–57%), followed by coronary artery disease (10–17%), and congestive heart failure (6–7%) [2,3]. Based on other reports, 30–35% of COVID-19 related deaths have underlying CVD, while the comorbid patients with CVD have an increased risk of severe manifestations of COVID-19 [4–7]. However, using retrospective analyses of 50 COVID-19 patients (mean age 64.80 ± 14.51 years) who were admitted to ICU, Aladag and Atabey demonstrated that pre-existing CVD is not a significant contributor in mortality with severe COVID-19 [8].

Nevertheless, cardiac injury has been reported in patients with COVID-19 having no history of cardiovascular issues [7]. National Health Commission of China reported that around 11.8% of patients died due to COVID-19-mediated onset of heart dysfunction during the course of illness [9]. Brit Long et al. evaluated 45 recent reports regarding COVID-19 and heart complications and confirmed the fatal outcome of COVID-19 on heart disablement even in patients with no pre-existing CVD record [7]. Notably, a SARS-CoV-2 positive 16-year-old boy developed acute myocarditis without showing any sign of COVID-19, except fever [10].

All these pieces of evidence prompted the establishment of the causal link of fatal co-morbidity of CVD and COVID-19, which might have caused the baffling overall prognosis of COVID-19 patients with or without CVD. Given this perplexing situation, it is important to analyse two basic assumptions whether: (1) the CVD pathologies favour SARS-CoV-2 and vice versa, and (2) the therapies meant for CVD are counterproductive to the treatment for COVID-19 and vice versa. Currently, the molecular evidence might indicate supporting both assumptions – hence clinicians and scientists are facing challenges to offer an effective treatment plan for comorbid patients with CVD and COVID-19.

However, the current knowledge on the CVD pathologies and pathogenesis of SARS-CoV-2 might shed a light on how Zinc (Zn) supplements might offer clinical benefits to this struggle.

Outline of the narrative review

To explain the potential benefits of the Zn supplement, the current review will first demonstrate that angiotensinogen converting enzyme 2 (ACE2) forms the central pathogenic link between CVD and COVID-19. This will then suggest a selective treatment plan to minimize the risk of cardiovascular pathology with COVID-19 comorbidity. The review will finally highlight possible beneficial role of Zn to inhibit SARS-CoV-2 replication as well as its role in the functional expression of ACE2- the receptor of SARS-CoV-2. Scientific rationale of the arguments established in the current review is based on the published papers that have described pathogenesis of CVD and COVID-19 as well as how these two diseases were reported during the ongoing pandemic.

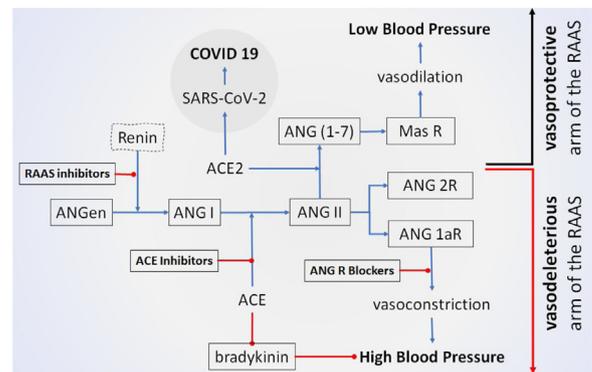


Fig. 1. COVID-19 is tied to create imbalance between vasoprotective and vasodeleterious arms of RAAS towards high blood pressure. ACE hydrolyzes inactive decapeptide ANG I to the octapeptide ANG II. ANG II binds to either ANG II receptor 1a (ANG 1aR) leading to tissue damage and lung edema, or to ANG II receptor 2 (ANG 2R) reducing tissue damage. Binding to ANG 1aR, ANG II causes vasoconstriction that results in hypertension. ACE2 on the other hand, can lower blood pressure by catalysing the hydrolysis of ANG II into a vasodilator ANG (1-7) thus counters the activity of the ACE by reducing the amount of ANG II and increasing ANG (1-7). While SARS-CoV-2 enters pulmonary cells by binding to ACE2, therefore, in case of SARS-CoV-2 infection, the therapeutic advantage to inhibit functional expression of ACE2 would favour vasodeleterious arm of the RAAS. On the other hand, therapeutic interventions to treat hypertensive patients should favour the vasoprotective arm i.e., towards the functional expression of ACE2. Hence, any individual with both SARS-CoV and hypertension would face a challenge to prioritize the treatment options. [→ = stimulation/activation; ● = inhibition/reduction].

Pathology of CVD and angiotensinogen converting enzyme

Cardiovascular diseases are a group of disorders of the heart and blood vessels resulting in heart attacks or strokes mainly caused by a blockage that prevents blood from flowing to the heart or brain. The cause of heart attacks and strokes is usually the presence of combined risk factors, including hypertension or high (elevated) blood pressure, diabetes, and hyperlipidaemia.

Physiological control of blood pressure is primarily a hormone-mediated system that maintains an equilibrium of fluid and electrolytes. The system starts with renin that carries out the conversion of angiotensinogen (ANGen) to angiotensin I (ANG I) [11]. Angiotensinogen converting enzyme (ACE) then hydrolyzes inactive decapeptide ANG I to the octapeptide ANG II by removing His-Leu residues from the C-terminal end [12]. The binding of ANG II to the type 1 ANG II receptor (ANG 1aR) results in vasoconstriction and therefore increased blood pressure. This cascade of events often is referred to as the vasodeleterious arm of RAAS (renin-angiotensinogen-aldosterone system) (Fig. 1). Angiotensin-converting enzyme 2 (ACE2) on the other hand, can lower blood pressure by catalyzing the hydrolysis of ANG II into vasodilator angiotensin (1-7). Thus, the activity of the ACE is balanced by reducing the amount of ANG-II and increasing ANG (1-7) which subsequently binds to Mas receptor (MAS R) causing vasodilation [13]. This cascade of events is referred to as the vasoprotective arm of RAAS (Fig. 1).

All these molecules are constitutively expressed in various tissues including the heart and lungs. Hence, a balance in the functional expression of those molecules ensures an optimum blood pressure (Fig. 1).

Pathology of SARS-CoV-2 infection starts with ACE-2

Using spike glycoproteins (S glycoproteins), SARS-CoV-2 particularly binds to ACE2 expressed in various organs of the body including lung alveolar and alveolar monocytes and macrophages [14–16]. It triggers imbalanced T cell activation against the virus hence a massive inflammatory cascade termed ‘cytokine storm’ is

ensued eventually [17]. This spurring immune response along with the viral multiplication leads to pulmonary cell destruction [18,19]. Consequently, the blood oxygen level drops, making the heart work harder and faster to pump blood throughout the body.

SARS-CoV-2 binding to ACE2 receptors triggers conformational changes in the S-glycoprotein. The virus is then endocytosed into the cytoplasm. Endosomal pH favours the host protease to cleave the S-glycoprotein resulting in the fusion of the viral envelope. Subsequently, the positive-strand viral genomic RNA is released into the cell cytoplasm. SARS-CoV-2 replication starts with RNA-dependent RNA polymerase (RdRp) which is integrated into a membrane-associated viral enzyme complex to allow the synthesis of negative-strand RNA. The negative RNA strand is used as a template for the synthesis of viral mRNA [20,21].

ACE2: the epicentre of CVD and COVID-19 pathogenesis

ACE2 is mostly bound to cell membranes of various organs of the body including the heart, blood vessels, gut, lung, kidney, testis, brain while only scarcely present in the circulation in a soluble form, as well as in alveolar monocytes and macrophages [14–16,22]. The ACE2 receptor, a transmembrane type I glycoprotein, was initially discovered by two independent groups in the year 2000 and has a 40% structural identity to ACE [23,24]. This monooxypeptidase has 805 amino acids with one extracellular catalytic domain that catalyses the removal of one amino acid from the C-terminal end of ANG II and converts it to ANG (1-7).

While ACE2 serves as the receptor for SARS-CoV-2 binding and subsequently entering host cells [15], *in vivo* studies revealed that lung ACE2 expression is markedly decreased upon SARS-CoV infection [22]. Only a handful of molecular evidence, as mentioned below, restricts to conclude convincingly - whether SARS-CoV-2, like its ancestor SARS-CoV, will lead to a decreased expression of ACE2 upon infecting the host cells. However, SARS-CoV and SARS-CoV-2 share more than 70% identity in the amino acid sequence [25], hence is not unlikely to see a reduced expression of ACE2 after SARS-CoV-2 infection.

In vivo experiment involving a rat model of acute respiratory distress syndrome has shown increased ACE activity and ANG II expression, while ACE2 activity and ANG (1-7) levels were reduced [26,27]. Furthermore, a negative correlation was shown between ACE2 expression and COVID-19 fatality at molecular levels [28]. Again, the SARS-CoV-2 genome was found in 7 out of 20 post-mortem autopsy of heart tissues that were characterized by increased myocardial fibrosis, inflammation, and reduced myocardial ACE2 expression [29].

At the same time, patients with CVDs have increased ACE2 as compared to healthy controls [7]. It is possible then; CVD patients might be more susceptible to SARS-CoV-2 attack due to more viral entry through binding to ACE2. The binding of SARS-CoV-2 to cardiac ACE2 can be assumed to influence two events concurrently: (1) hindering the vasoprotective arm of RAAS by retarding the conversion of ANG II to ANG (1-7) occurring in heart tissues [30], and (2) aggravating the vasodeleterious arm of the RAAS by allowing the conversion of ANG II to ANG 1aR culminating to hypertension. Overall, these lines of evidence place SARS-CoV-2 as a double-edged sword involving the ACE2 at the epicentre of the pathogenesis (Fig. 2).

This might be the reason for higher cardiovascular damage during COVID-19 progression [2–7,31]. This can be reasonably argued based on the observation that ACE2 becomes depleted due to SARS-CoV-2 infection, which otherwise could convert ANG II into ANG (1-7), but not ANG 1aR, the vasoconstriction consequently results.

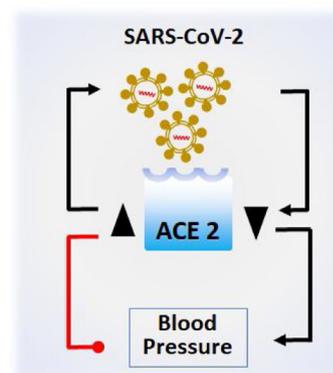


Fig. 2. ACE2 is at the centre of COVID-19 and CVD. Increased ACE2 expression (▲) would allow more SARS-CoV-2 to enter host cells by binding to ACE2, while SARS-CoV-2 infection results in the decreased expression of ACE2 (▼). Increased ACE2 expression (▲) on the other hand, reduces blood pressure, while the decreased ACE2 expression (▼) would result in higher blood pressure [→ = stimulation/increase; → = inhibition/reduction].

Impact of CVD treatment in COVID-19 comorbidity

A range of medications is prescribed for patients with hypertension to prevent heart attacks and strokes. Among them, Angiotensin-converting enzyme (ACE) inhibitors, Angiotensin II receptor blockers (ARB), and other renin-angiotensin-aldosterone system (RAAS) inhibitors are the drugs that interrupt different steps in the abnormally activated RAAS system responsible for elevated blood pressure in the human body.

An uncontrolled RAAS activation results in continuous vasoconstriction and hypertension. As a method of restoration, medications like ACE inhibitors are used to relax blood vessels by blocking the formation of ANG II that narrows blood vessels [32] while ARB helps relax blood vessels by blocking the action of ANG II on its receptors [33]. Both ACE inhibitors and ARBs can reduce angiotensin II levels [33]. In general, ACE inhibitors reduce the substrate of ANG II generation, and the ARBs arrest ANG 1aR activity to counter vasoconstriction. RAAS inhibitors on the other hand, slow down the production of renin hormone from the kidney that starts the RAAS [34].

The antihypertensive agent used to treat high blood-pressure patients including ARBs, ACE inhibitors, and RAAS inhibitors can upregulate ACE2 expression in rodent studies, hence is suspected to potentially increase viral entry sites for coronaviruses worsening the outcome in patients with COVID-19 [35].

However, different RAAS inhibitors have different effects on ACE2 levels- the key for SARS-CoV-2 to enter cells for its replication. ACE inhibitor (captopril) caused a significant increase in ACE2 protein expression in rats with acute lung injury [36]. While ACE2 has been reported to have protective effects in acute lung injury [37]. Contrarily, other ACE inhibitors (lisinopril) and ARB (losartan) alone or in combination did not increase cardiac ACE2 activity but caused a significant increase in ACE2 mRNA expression. However, lisinopril caused a 1.8-fold increase in rat plasma ANG-(1-7) and decreased plasma ANG II. Losartan, on the other hand, increased plasma levels of both ANG-(1-7) and ANG II, with increased cardiac ACE2 mRNA and concomitant cardiac ACE2 activity [38]. Therapeutic intervention with the cyclic form of ANG-(1-7) attenuated the inflammatory mediator response, markedly decreased lung injury scores, and increased oxygenation [26].

The above-mentioned evidence demonstrates that therapeutic interventions for CVD involving certain ACE inhibitors would increase the functional expression of ACE2. Continuation of CVD treatment using such inhibitors with COVID-19 comorbidity might have two possible outcomes of opposing spectrum: (1) favouring

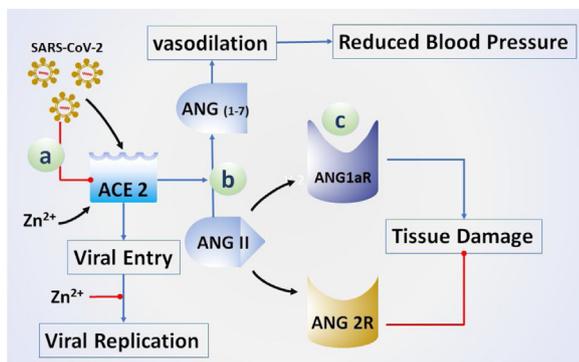


Fig. 3. Potential sites of drug target and Zn action to favour both CVD and COVID-19 treatments. Zn supplement might aid to activate or upregulate functional ACE2 expression countering SARS-CoV-2 mediated ACE2 downregulation (indicated with a). Zn can also inhibit SARS-CoV-2 replication by inhibiting RdRp. The second possible target might aid to increase the conversion of ANGII to ANG (1-7) to favour vasodilatory impact (indicated with b). The third possible therapeutic target might focus on the antagonist to ANG II to bind ANG 1aR (indicated with c). [→ = stimulation/activation; → = inhibition or reduction].

SARS-CoV-2 to enter host cells resulting in fatal consequences for COVID-19 patients, or (2) improving cardiovascular conditions by prompting functional expression of ACE2 that was reduced as a result of SARS-CoV-2 infection.

The latter outcome could be beneficial based on the observation of a reduced ACE2 expression in the lungs in experimental SARS-CoV infections of wild-type mice. That in turn suggests that a reduced ACE2 expression might have a role in SARS-CoV-mediated severe acute lung pathologies. At the same time, SARS-CoV Spike protein binding to ACE2 in cell lines or SARS-CoV infections *in vivo* results in reduced ACE2 protein expression [22]. A deficiency of ACE2 in mice results in a dramatic decrease in viral replication and much less severe pathologic alterations in lungs as compared to wild-type mice [22,37].

On the other hand, ACE inhibitors that have no impact on the functional expression of ACE2 which could favour the vasoprotective arm of RAAS to reduce high blood pressure might be futile for the patients with COVID-19, since SARS-CoV-2 infection reduces ACE2 expression.

Notably, ACE2 is greatly expressed in epithelial cells of alveoli, trachea, bronchi, bronchial serous glands [16], and alveolar monocytes and macrophages, as well as in coronary vessels along with cardiac myocytes and fibroblasts [39].

Alternative treatment plans for CVD and COVID-19 comorbidity

Given the above discussion, it might be advisable to consider a treatment plan for COVID-19 patients with CVD in an approach that would neither aggravate the vasodeleterious arm of RAAS nor compromise the vasoprotective arm of RAAS which at the same time would minimize or if possible inhibit viral (SARS-CoV-2) replication.

First, this would require activation of ACE2. Because ACE2 can convert ANG II to ANG (1-7) and eventually can cause vasodilation. However, SARS-CoV-2 infection results in the downregulation of ACE2, hence an additional Zn supplement might aid to activate or upregulate functional ACE2 expression. This can be reasonably argued based on the fact that ACE2 is a zinc-containing metalloenzyme [40] (Fig. 3, indicated with the letter 'a').

The second possible target might aid to increase the conversion of ANGII to ANG (1-7) as this has vasodilatory impact hence would reduce the blood pressure (Fig. 3, indicated with the letter 'b'). In

the same line of direction, RAAS inhibitors were suggested to be beneficial in COVID-19 treatment [41].

The third possible therapeutic target might focus on the reduction of the binding of the ANGII with angiotensin II receptor 1a (ANG1aR) as this binding aggravates the tissue damage. This could be achieved by designing any antagonist to ANG II to bind ANG 1aR. (Fig. 3, indicated with the letter 'c'). In favour of this notion, it can be highlighted that COVID-19 patients once given angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type 1 receptor blockers (ARBs) showed reduced severity and a lower level of IL-6 in peripheral blood which in turn also increased CD3 and CD8 T cell counts in peripheral blood and decreased the peak viral load compared to other antihypertensive drugs [42].

How Zn²⁺ supplement would offer a beneficial role in CVD and COVID-19 comorbidity?

In biological fluids, zinc ion (Zn²⁺) works as a Lewis acid hence acts as a stable cofactor in redox reactions occurring in cellular and extracellular environment [43]. Zinc is directly involved in cell-mediated immunity against infectious bacteria and viruses [44–47]. For example, cell specific impact of Zn deficiency may result in a decreased activation of NF-κB that in turn cause reduced IL-2 and IL-2 receptor-α in T helper cell line. Thus, a potential therapeutic roles of Zn was suggested in acute infantile diarrhea, acrodermatitis enteropathica, blindness in patients with age-related macular degeneration, and the treatment of common cold [44]. In addition, *in vivo*, zinc deficiency negatively impact immunological functions of granulocytes, monocytes, NK-, T-, and B-cells. Regulatory role of free Zn²⁺ in signal transduction in cells of the immune system involves several molecular targets, such as phosphatases, phosphodiesterases, caspases, and kinases [45].

Again, increased Zn at the site of infection causing upregulated expression of Zn importing proteins (i.e., ZIP) by the circulating leukocytes or infiltrated inflammatory cells, induces expression of metallothionein (MT) which not only protect leukocytes from the increased influx of Zn at the site of infections but also helps those cells to fight against the infection [47].

Furthermore, Zn is involved in the proliferation of neutrophils, NK cells, macrophages, and T and B lymphocytes as well as their immune functions such as cytokine production. Zn also mediates protection against reactive oxygen species (ROS) that are generally produced during inflammatory processes. Free intracellular Zn²⁺ is essential in extravasation to the site of the infection and uptake and killing of microorganisms by neutrophils [48].

Activation of immune responses such as NF-κB signalling requires Zn²⁺. Notably, NF-κB regulates the expression of pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α, chemokines, acute phase proteins, matrix metalloproteinases, adhesion molecules, growth factors, and other factors involved in the inflammatory response, such as COX-2 and iNOS [49,50]. Zinc administration in mixed lymphocyte cultures induces and stabilizes subsets of CD4⁺ T cells [51], while CD4⁺ T-cells, commonly known as helper T-cells, play critical roles in antiviral immunity [52,53]. Zn²⁺ also serves as an intracellular second messenger and triggers apoptosis or a decrease in protein synthesis at elevated concentrations [54–56].

Zn also plays a critical role in autophagy under basal conditions - a natural, controlled mechanism to remove unnecessary or dysfunctional components from within the cells [57]. This notion was supported by an *in vitro* experiment using human hepatoma cells, VL-17A where Zn depletion caused a significant suppression of autophagy; on the contrary, an addition of the element to the culture enhanced autophagy [57]. Notably, in autophagy, the intracellular components such as protein aggregates and damaged

organelles are degraded by lysosomal enzymes [58,59] including proteases, peptidases, phosphatases, nucleases, glycosidases, sulfatases, and lipases [60] – while structural and functional integrity of many of these enzymes depends on Zn [61]. In fact, during viral infections, autophagy provides protection as a defence mechanism by auto-degradation of the infected cells [62–64].

As a Zn-metalloenzyme, the functional expression of ACE2 [40] is expected to be increased by Zn²⁺ supplement. At the same time, Zn²⁺ is expected to inhibit SARS-CoV-2 replication as Zn²⁺ was reported to inhibit the *in vitro* RNA-dependent RNA polymerase (RdRp) activity by inhibiting the SARS-CoV RdRp elongation and template binding [65].

Earlier it was also shown that Zn²⁺ inhibited the proteolytic processing of replicase polyproteins [66]. To allow Zn²⁺ to exert its inhibitory effect on SARS-CoV-2 viral replication, Zn²⁺ entry inside the cell might be enhanced by ionophores such as dithiocarbamates [67], pyrithione [65,68], zincophorin [69], and hydroxychloroquine [65,70,71]. It can be noted that a meta-analysis involving 19 reported studies suggested that chloroquine/hydroxychloroquine was associated with a reduced risk of CVD in patients with rheumatic diseases [72].

The ability of Zn²⁺ to inhibit the replication of various RNA viruses has been demonstrated in a good number of *in vitro* studies. For example, in the presence of its cellular import stimulatory compounds such as hinokitol (HK), pyrrolidine dithiocarbamate (PDTC), and pyrithione (PT), the added Zn²⁺ inhibited the replication of influenza virus [73], respiratory syncytial virus [74], and several picornaviruses [71,75,76]. Their interference with polyprotein processing in cells infected with human rhinovirus and coxsackievirus B3 is well evidenced [71].

The other modes of action that Zn salts exhibit to inhibit SARS-CoV-2 as well as other viruses, viz. HIV, HSV, and vaccinia virus are inhibition of the viral entry, blocking of polyprotein processing, and inhibition of viral RdRp activity [65,77,78]. Zn salts namely Zn-sulfate and Zn-acetate were also shown to inhibit viral sense and antisense RNA levels by approximately 50%, thus inhibiting viral replication [79].

A retrospective case series study confirmed that triple therapy involving Zn, low-dose HCQ, and azithromycin as early as possible after symptom onset results in significantly fewer hospitalisations [80]. In the same line of direction, a multi-centre cohort study revealed an association between Zn supplement with an ionophore, and both increased rates of discharge home and a 24% reduced risk of in-hospital mortality among COVID-19 patients [81]. Again, adequate intake of Zn, vitamins C, and D was earlier suggested to combat the SARS-CoV-2 infection [82]. However, in a randomized clinical trial involving 214 adult COVID-19 patients, a high-dose of Zn with vitamin C (ascorbic acid) supplement was found ineffective to alleviate symptoms of the disease [83].

In addition to those direct effects on the virus as well as to improve the clinical outcome of CVD treatment, several immunome pathways such as the NF-κB signalling pathway are activated by Zn²⁺ [84]. This might control cytokine storm in COVID-19 patients by regulating the expression of pro-inflammatory cytokines namely IL-1b, IL-6, IL-8, TNF-α, and MCP-1; chemokines, acute phase proteins, matrix metalloproteinases, adhesion molecules, growth factors, as well as COX-2 and iNOS [49,50].

Conclusion

COVID positive patients' cardiac health, with or without respiratory symptoms could be evaluated for resolving primary complications of COVID-19 and to reduce mortality from potential cardiovascular conditions. Based on the patients' condition, a selective treatment plan might be required to ensure minimal

lung injury or cardiovascular pathologies. Special attention might be required to influence the functional expression of ACE2. Given the above discussion on the involvement of Zn²⁺, its use as a supplement with an appropriate ionophore would offer multiple benefits to CVD and COVID-19 comorbid patients: (1) preventing viral replication by inhibiting the RdRp of the SARS-CoV-2, (2) enhance protective immune responses, and (3) restoring the functional balance of ACE2.

Conflict of interest statement

All authors declare that there is no conflict of interest.

Acknowledgement

No funds were available for this review work.

References

- [1] Vrints CJM, Krychtiuk KA, Van Craenenbroeck EM, Segers VF, Price S, Heidebuchel H. Endothelialitis plays a central role in the pathophysiology of severe COVID-19 and its cardiovascular complications. *Acta Cardiol* 2020;1–16, <http://dx.doi.org/10.1080/00015385.2020.1846921>.
- [2] Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical Characteristics of Covid-19 in New York City. *N Engl J Med* 2020;100:1–3, <http://dx.doi.org/10.1056/NEJMc2005203>.
- [3] Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes among 5700 Patients Hospitalized with COVID-19 in the New York City Area. *J Am Med Assoc* 2020;323:2052–9, <http://dx.doi.org/10.1001/jama.2020.6775>.
- [4] Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *J Am Med Assoc* 2020;323:1775–6, <http://dx.doi.org/10.1001/jama.2020.4683>.
- [5] Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular Implications of Fatal Outcomes of Patients with Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol* 2020;2019:811–8, <http://dx.doi.org/10.1001/jamacardio.2020.1017>.
- [6] Wang L, He W, Yu X, Hu D, Bao M, Liu H, et al. Coronavirus Disease 2019 in elderly patients: characteristics and prognostic factors based on 4-week follow-up. *J Infect* 2020, <http://dx.doi.org/10.1016/j.jinf.2020.03.019>.
- [7] Long B, Brady WJ, Koyfman A, Gottlieb M. Cardiovascular complications in COVID-19. *Am J Emerg Med* 2020;38:1504–7, <http://dx.doi.org/10.1016/j.ajem.2020.04.048>.
- [8] Aladağ N, Atabey RD. The role of concomitant cardiovascular diseases and cardiac biomarkers for predicting mortality in critical COVID-19 patients. *Acta Cardiol* 2020;1–8, <http://dx.doi.org/10.1080/00015385.2020.1810914>.
- [9] Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and Cardiovascular Disease. *Circulation* 2020;2019:1648–55, <http://dx.doi.org/10.1161/CIRCULATIONAHA.120.046941>.
- [10] Gnechchi M, Moretti F, Bassi EM, Leonardi S, Totaro R, Perotti L, et al. Myocarditis in a 16-year-old boy positive for SARS-CoV-2. *Lancet* 2020;395:e116, [http://dx.doi.org/10.1016/S0140-6736\(20\)31307-6](http://dx.doi.org/10.1016/S0140-6736(20)31307-6).
- [11] Kumar R, Singh VP, Baker KM. The intracellular renin-angiotensin system in the heart. *Curr Hypertens Rep* 2009;11:104, <http://dx.doi.org/10.1007/s11906-009-0020-y>.
- [12] Coates D. The angiotensin converting enzyme (ACE). *Int J Biochem Cell Biol* 2003;35:769–73, [http://dx.doi.org/10.1016/S1357-2725\(02\)00309-6](http://dx.doi.org/10.1016/S1357-2725(02)00309-6).
- [13] Rodrigues Prestes TR, Rocha NP, Miranda AS, Teixeira AL, Simoes-E-Silva AC. The anti-inflammatory potential of ACE2/angiotensin-(1-7)/Mas receptor axis: Evidence from basic and clinical research. *Curr Drug Targets* 2017;18:1301–13, <http://dx.doi.org/10.2174/1389450117666160727142401>.
- [14] Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004;203:631–7, <http://dx.doi.org/10.1002/path.1570>.
- [15] Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, 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, <http://dx.doi.org/10.1038/s41467-020-15562-9>.
- [16] Liu L, Wei Q, Alvarez X, Wang H, Du Y, Zhu H, 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;85:4025–30, <http://dx.doi.org/10.1128/JVI.02292-10>.
- [17] Wang L, Zhang Y, Zhang S. Cardiovascular impairment in COVID-19: Learning from current options for cardiovascular anti-inflammatory therapy. *Front Cardiovasc Med* 2020;7:78, <http://dx.doi.org/10.3389/fcvm.2020.00078>.
- [18] Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* 2020;181:1036–45.e9, <http://dx.doi.org/10.1016/j.cell.2020.04.026>.
- [19] Schwartz MD, Emerson SG, Punt J, Goff WD. Decreased Naïve T-cell Production Leading to Cytokine Storm as Cause of Increased COVID-19 Severity with

- Comorbidities. *Aging Dis* 2020;11:742–5, <http://dx.doi.org/10.14336/AD.2020.0619>.
- [20] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* 2015;1282:1–23, http://dx.doi.org/10.1007/978-1-4939-2438-7_1.
- [21] Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. *Nat Rev Microbiol* 2009;7:439–50, <http://dx.doi.org/10.1038/nrmicro2147>.
- [22] Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005;11:875–9, <http://dx.doi.org/10.1038/nm1267>.
- [23] Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme: Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000;275:33238–43, <http://dx.doi.org/10.1074/jbc.M002615200>.
- [24] Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res* 2000;87:E1–9, <http://dx.doi.org/10.1161/01.res.87.5.e1>.
- [25] Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci* 2020;63:457–60, <http://dx.doi.org/10.1007/s11427-020-1637-5>.
- [26] Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1–7) or an angiotensin II receptor antagonist. *J Pathol* 2011;225:618–27, <http://dx.doi.org/10.1002/path.2987>.
- [27] Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med* 2020;76:14–20, <http://dx.doi.org/10.1016/j.ejim.2020.04.037>.
- [28] Chen J, Jiang Q, Xia X, Liu K, Yu Z, Tao W, et al. Individual variation of the SARS-CoV-2 receptor ACE2 gene expression and regulation. *Aging Cell* 2020;19, <http://dx.doi.org/10.1111/acel.13168>.
- [29] Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest* 2009;39:618–25, <http://dx.doi.org/10.1111/j.1365-2362.2009.02153.x>.
- [30] Wang Y, Roeber L, Tse G, Liu T. 2019–Novel Coronavirus-Related Acute Cardiac Injury Cannot Be Ignored. *Curr Atheroscler Rep* 2020;22:2019–20, <http://dx.doi.org/10.1007/s11883-020-00842-y>.
- [31] South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. *Am J Physiol Heart Circ Physiol* 2020;318:H1084–90, <http://dx.doi.org/10.1152/ajpheart.00217.2020>.
- [32] Dzau VJ. Mechanism of Action of Angiotensin-Converting Enzyme (ACE) Inhibitors in Hypertension and Heart Failure. *Drugs* 1990;39:11–6, <http://dx.doi.org/10.2165/00003495-199000392-00004>.
- [33] Mirabito Colafella KM, Bovée DM, Danser AHJ. The renin-angiotensin-aldosterone system and its therapeutic targets. *Exp Eye Res* 2019;186:107680, <http://dx.doi.org/10.1016/j.exer.2019.05.020>.
- [34] Gradman AH, Schmieder RE, Lins RL, Nussberger J, Chiang Y, Bedigian MP, Aliskiren, a novel orally effective renin inhibitor, provides dose-dependent antihypertensive efficacy and placebo-like tolerability in hypertensive patients. *Circulation* 2005;111:1012–8, <http://dx.doi.org/10.1161/01.CIR.0000156466.02908.ED>.
- [35] Nicholls J, Peiris M. Good ACE, bad ACE do battle in lung injury, SARS. *Nat Med* 2005;11:821–2, <http://dx.doi.org/10.1038/nm0805-821>.
- [36] Li Y, Zeng Z, Li Y, Huang W, Zhou M, Zhang X, et al. Angiotensin-converting enzyme inhibition attenuates lipopolysaccharide-induced lung injury by regulating the balance between angiotensin-converting enzyme and angiotensin-converting enzyme 2 and inhibiting mitogen-activated protein kinase activation. *Shock* 2015;43:395–404, <http://dx.doi.org/10.1097/SHK.0000000000000302>.
- [37] Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;436:112–6, <http://dx.doi.org/10.1038/nature03712>.
- [38] Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 2005;111:2605–10, <http://dx.doi.org/10.1161/CIRCULATIONAHA.104.510461>.
- [39] Varagic J, Ahmad S, Nagata S, Ferrario CM. ACE2: angiotensin II/angiotensin-(1–7) balance in cardiac and renal injury. *Curr Hypertens Rep* 2014;16:420, <http://dx.doi.org/10.1007/s11906-014-0420-5>.
- [40] Turner AJ. ACE2 Cell Biology, Regulation, and Physiological Functions. In: *Prot. Arm Renin Angiotensin Syst. Funct. Asp. Ther. Implic.* Elsevier Inc.; 2015. p. 185–9, <http://dx.doi.org/10.1016/B978-0-12-801364-9.00025-0>.
- [41] Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc Res* 2020;116:1666–87, <http://dx.doi.org/10.1093/cvr/cvaa106>.
- [42] Meng J, Xiao G, Zhang J, He X, Ou M, Bi J, et al. Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg Microbes Infect* 2020;9:757–60, <http://dx.doi.org/10.1080/22221751.2020.1746200>.
- [43] Baś S, Szewczyk Marcin, Mlynarski J. Zinc-based Chiral Lewis Acids. In: *Mlynarski J, editor. Chiral Lewis Acids Org. Synth.* Wiley Online Library; 2017., <http://dx.doi.org/10.1002/9783527802142.ch5>.
- [44] Prasad AS. Zinc in human health: effect of zinc on immune cells. *Mol Med* 2008;14:353–7, <http://dx.doi.org/10.2119/2008-00033.Prasad>.
- [45] Haase H, Rink L. Zinc signals and immune function. *Biofactors* 2014;40:27–40, <http://dx.doi.org/10.1002/biof.1114>.
- [46] Bonaventura P, Benedetti G, Albareda F, Miossec P. Zinc and its role in immunity and inflammation. *Autoimmun Rev* 2015;14:277–85, <http://dx.doi.org/10.1016/j.autrev.2014.11.008>.
- [47] Rahman MT, Karim MM. Metallothionein: a Potential Link in the Regulation of Zinc in Nutritional Immunity. *Biol Trace Elem Res* 2018;182:1–13, <http://dx.doi.org/10.1007/s12011-017-1061-8>.
- [48] 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, <http://dx.doi.org/10.1007/s12011-015-0515-0>.
- [49] Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol* 2009;1:a001651, <http://dx.doi.org/10.1101/cshperspect.a001651>.
- [50] Hayden MS, Ghosh S. Regulation of NF-kappaB by TNF family cytokines. *Semin Immunol* 2014;26:253–66, <http://dx.doi.org/10.1016/j.smim.2014.05.004>.
- [51] Maywald M, Rink L. Zinc supplementation induces CD4(+)/CD25(+)/Foxp3(+) antigen-specific regulatory T cells and suppresses IFN-gamma production by upregulation of Foxp3 and KLF-10 and downregulation of IRF-1. *Eur J Nutr* 2017;56:1859–69, <http://dx.doi.org/10.1007/s00394-016-1228-7>.
- [52] Whitmire JK, Ahmed R. Costimulation in antiviral immunity: differential requirements for CD4(+) and CD8(+) T cell responses. *Curr Opin Immunol* 2000;12:448–55, [http://dx.doi.org/10.1016/S0952-7915\(00\)00119-9](http://dx.doi.org/10.1016/S0952-7915(00)00119-9).
- [53] Jansen JM, Gerlach T, Elbaheesh H, Rimmelzwaan GF, Saletti G. Influenza virus-specific CD4+ and CD8+ T cell-mediated immunity induced by infection and vaccination. *J Clin Virol* 2019;119:44–52, <http://dx.doi.org/10.1016/j.jcv.2019.08.009>.
- [54] Alirezai M, Nairn AC, Glowinski J, Premont J, Marin P. Zinc inhibits protein synthesis in neurons. Potential role of phosphorylation of translation initiation factor-2alpha. *J Biol Chem* 1999;274:32433–8, <http://dx.doi.org/10.1074/jbc.274.45.32433>.
- [55] Frederickson CJ, Koh J-Y, Bush AI. The neurobiology of zinc in health and disease. *Nat Rev Neurosci* 2005;6:449–62, <http://dx.doi.org/10.1038/nrn1671>.
- [56] Lazarczyk M, Favre M. Role of Zn²⁺ ions in host–virus interactions. *J Virol* 2008;82:11486–94, <http://dx.doi.org/10.1128/JVI.01314-08>.
- [57] Liuzzi JP, Yoo C. Role of zinc in the regulation of autophagy during ethanol exposure in human hepatoma cells. *Biol Trace Elem Res* 2013;156:350–6, <http://dx.doi.org/10.1007/s12011-013-9816-3>.
- [58] Mizushima N. A brief history of autophagy from cell biology to physiology and disease. *Nat Cell Biol* 2018;20:521–7, <http://dx.doi.org/10.1038/s41556-018-0092-5>.
- [59] Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell* 2019;176:11–42, <http://dx.doi.org/10.1016/j.cell.2018.09.048>.
- [60] Lubke T, Lobel P, Sleat DE. Proteomics of the lysosome. *Biochim Biophys Acta* 2009;1793:625–35, <http://dx.doi.org/10.1016/j.bbamcr.2008.09.018>.
- [61] Maret W. Zinc biochemistry: from a single zinc enzyme to a key element of life. *Adv Nutr* 2013;4:82–91, <http://dx.doi.org/10.3945/an.112.003038>.
- [62] Jiang P, Mizushima N. Autophagy and human diseases. *Cell Res* 2014;24:69–79, <http://dx.doi.org/10.1038/cr.2013.161>.
- [63] Choi AMK, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med* 2013;368:651–62, <http://dx.doi.org/10.1056/NEJMr1205406>.
- [64] Meijer AJ, Codogno P. Autophagy: regulation and role in disease. *Crit Rev Clin Lab Sci* 2009;46:210–40, <http://dx.doi.org/10.1080/10408360903044068>.
- [65] te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog* 2010;6:e1001176, <http://dx.doi.org/10.1371/journal.ppat.1001176>.
- [66] Denison MR, Zoltick PW, Hughes SA, Giangreco B, Olson AL, Perlman S, et al. Intracellular processing of the N-terminal ORF 1a proteins of the coronavirus MHV-A59 requires multiple proteolytic events. *Virology* 1992;189:274–84, [http://dx.doi.org/10.1016/0042-6822\(92\)90703-r](http://dx.doi.org/10.1016/0042-6822(92)90703-r).
- [67] Kim CH, Kim JH, Hsu CY, Ahn YS. Zinc is required in pyrrolidine dithiocarbamate inhibition of NF-kB activation. *FEBS Lett* 1999;449:28–32, [http://dx.doi.org/10.1016/S0014-5793\(99\)00390-7](http://dx.doi.org/10.1016/S0014-5793(99)00390-7).
- [68] Kim CH, Kim JH, Moon SJ, Chung KC, Hsu CY, Seo JT, et al. Pyrrithione, a Zinc ionophore, Inhibits NF-kB Activation. *Biochem Biophys Res Commun* 1999;259:505–9, <http://dx.doi.org/10.1006/bbrc.1999.0814>.
- [69] Brooks HA, Gardner D, Poyser JP, King TJ. The structure and absolute stereochemistry of zincophorin (antibiotic M144255): a monobasic carboxylic acid ionophore having a remarkable specificity for divalent cations. *J Antibiot (Tokyo)* 1984;37:1501–4, <http://dx.doi.org/10.17164/antibiotics.37.1501>.
- [70] Xue J, Moyer A, Peng B, Wu J, Hannafon BN, Ding W-Q. Chloroquine is a zinc ionophore. *PLoS One* 2014;9:e109180, <http://dx.doi.org/10.1371/journal.pone.0109180>.
- [71] Krenn BM, Gaudernak E, Holzer B, Lanke K, Van Kuppeveld FJM, Seipelt J. Antiviral activity of the zinc ionophores pyrithione and hinokitiol against picornavirus infections. *J Virol* 2009;83:58–64, <http://dx.doi.org/10.1128/JVI.01543-08>.
- [72] Liu D, Li X, Zhang Y, Kwong JS-W, Li L, Zhang Y, et al. Chloroquine and hydroxychloroquine are associated with reduced cardiovascular risk: a systematic review

- and meta-analysis. *Drug Des Devel Ther* 2018;12:1685–95, <http://dx.doi.org/10.2147/DDDT.S166893>.
- [73] Uchide N, Ohshima K, Bessho T, Yuan B, Yamakawa T. Effect of antioxidants on apoptosis induced by influenza virus infection: inhibition of viral gene replication and transcription with pyrrolidine dithiocarbamate. *Antiviral Res* 2002;56:207–17, [http://dx.doi.org/10.1016/s0166-3542\(02\)00109-2](http://dx.doi.org/10.1016/s0166-3542(02)00109-2).
- [74] Suara RO, Crowe JEJ. Effect of zinc salts on respiratory syncytial virus replication. *Antimicrob Agents Chemother* 2004;48:783–90, <http://dx.doi.org/10.1128/aac.48.3.783-790.2004>.
- [75] Korant BD, Kauer JC, Butterworth BE. Zinc ions inhibit replication of rhinoviruses. *Nature* 1974;248:588–90, <http://dx.doi.org/10.1038/248588a0>.
- [76] Lanke K, Krenn BM, Melchers WJG, Seipelt J, van Kuppeveld FJM. PDTC inhibits picornavirus polyprotein processing and RNA replication by transporting zinc ions into cells. *J Gen Virol* 2007;88:1206–17, <http://dx.doi.org/10.1099/vir.0.82634-0>.
- [77] Katz E, Margalith E. Inhibition of vaccinia virus maturation by zinc chloride. *Antimicrob Agents Chemother* 1981;19:213–7, <http://dx.doi.org/10.1128/aac.19.2.213>.
- [78] Haraguchi Y, Sakurai H, Hussain S, Anner BM, Hoshino H. Inhibition of HIV-1 infection by zinc group metal compounds. *Antiviral Res* 1999;43:123–33, [http://dx.doi.org/10.1016/s0166-3542\(99\)00040-6](http://dx.doi.org/10.1016/s0166-3542(99)00040-6).
- [79] Kaushik N, Subramani C, Anang S, Muthumohan R, Shalimar, Nayak B, et al. Zinc Salts Block Hepatitis E Virus Replication by Inhibiting the Activity of Viral RNA-Dependent RNA Polymerase. *J Virol* 2017;91, <http://dx.doi.org/10.1128/JVI.00754-17>.
- [80] Derwand R, Scholz M, Zelenko V. COVID-19 outpatients: early risk-stratified treatment with zinc plus low-dose hydroxychloroquine and azithromycin: a retrospective case series study. *Int J Antimicrob Agents* 2020;56:106214, <http://dx.doi.org/10.1016/j.ijantimicag.2020.106214>.
- [81] Frontera JA, Rahimian JO, Yaghi S, Liu M, Lewis A, de Havenon A, et al. Treatment with Zinc is Associated with Reduced In-Hospital Mortality Among COVID-19 Patients: A Multi-Center Cohort Study. *Res Sq* 2020, <http://dx.doi.org/10.21203/rs.3.rs-94509/v1>, rs.3.rs-94509.
- [82] Name JJ, Souza ACR, Vasconcelos AR, Prado PS, Pereira CPM. Zinc, Vitamin D and Vitamin C: Perspectives for COVID-19 With a Focus on Physical Tissue Barrier Integrity. *Front Nutr* 2020;7:606398, <http://dx.doi.org/10.3389/fnut.2020.606398>.
- [83] Thomas S, Patel D, Bittel B, Wolski K, Wang Q, Kumar A, et al. Effect of High-Dose Zinc and Ascorbic Acid Supplementation vs Usual Care on Symptom Length and Reduction Among Ambulatory Patients With SARS-CoV-2 Infection The COVID A to Z Randomized Clinical Trial; 2021. p. 1–10, <http://dx.doi.org/10.1001/jamanetworkopen.2021.0369>.
- [84] Kim YM, Cao D, Reed W, Wu W, Jaspers I, Tal T, et al. Zn²⁺-induced NF-κB-dependent transcriptional activity involves site-specific p65/RelA phosphorylation. *Cell Signal* 2007;19:538–46, <http://dx.doi.org/10.1016/j.cellsig.2006.08.003>.