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Authors: Muhammad Karim[1], Shahnaz Sultana[2], Rokaia Sultana[3], Mohammad Tariqur Rahman[4]

Affiliations: Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh[1], Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), New Elephant Road, Dhaka 1205, Bangladesh[2], Faculty of Dentistry, University of Malaya, Kuala Lumpur 50603, Malaysia[3]

Orcid ids: 0000-0003-0476-8239[1], 0000-0001-8845-2578[2], 0000-0002-2066-7617[3], 0000-0003-3600-3265[4]

Contact e-mail: manjur@du.ac.bd

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Potential role of Zinc supplement in CVD and COVID-19 co-morbidity

Muhammad Manjurul Karim¹, Shahnaz Sultana², Rokaia Sultana², and Mohammad Tariqur Rahman³

Affiliation

¹ Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh

² Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), New Elephant Road, Dhaka 1205, Bangladesh

³ Faculty of Dentistry, University of Malaya, Kuala Lumpur 50603, Malaysia

E-mail addresses:

MMK: manjur@du.ac.bd

SS: shahnazsultana339@gmail.com

RS: rokaiasultana@rocketmail.com

MTR: tarique@um.edu.my

*Correspondence: Mohammad Tariqur Rahman

ORCID ID:

MMK: 0000-0003-0476-8239 | SS: 0000-0001-8845-2578 | RS: 0000-0002-2066-7617 |

MTR: 0000-0003-3600-3265

Abstract

As far as the 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 RAAS nor compromise the vasoprotective arm of RAAS but is effective to minimize or if possible, inhibit the viral replication. A zinc supplement to the selective treatment plan, to be decided by the clinicians depending on the cardiovascular conditions of the patients, is hereafter proposed that might greatly enhance the therapeutic outcome. Notably, ACE2 is a zinc metalloenzyme and zinc is also known to inhibit viral replication.

Keywords: Angiotensin converting enzyme; Comorbidity; high blood pressure; vasodilation; vasoconstriction; SARS-COV-2; Zinc

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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.

As far as the 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%) [1–4]. Based on other reports, 30-35% of COVID-19 related deaths have underlying CVD while the comorbid patients with CVD have increased risk of severe manifestations of COVID-19 [5–8].

Cardiac injury has also been reported in patients with Covid-19 having no history on cardiovascular issues [8]. 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 ended up confirming the fatal outcome of coronavirus on heart disablement even in patients with no pre-existing CVD record [8]. Another investigation exhibited a SARS-CoV-2 positive 16-year-old boy, developed acute myocarditis without showing any sign of COVID-19, except fever [10].

All these evidences prompted to establish the causal link between 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 favor 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 to confirm any or both assumptions are in scanty – hence clinicians and scientists are facing challenges to find 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 to hypothesize a possible solution to this struggle.

The current review will demonstrate that angiotensin converting enzyme 2 (ACE2) forms the central pathogenic link between COVID-19 and CVD. That warrants a selective treatment plan to minimize the risk of cardio-vascular pathology in relation to COVID-19. The review will also highlight the beneficial role of Zn as a potential supplement to minimize the severity of the coexistence of CVD and COVID-19.

Pathology of CVD

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 are usually the presence of combined risk factors, including hypertension or high (elevated) blood pressure, diabetes, and hyperlipidaemia [11].

Physiological control of blood pressure is primarily a hormone-mediated system that maintains an equilibrium of fluid and electrolytes [12]. The system starts with rennin that carries out the conversion of angiotensinogen to angiotensin I (ANG I) [13]. Angiotensin converting enzyme (ACE) then hydrolyzes inactive decapeptide ANG I to the octapeptide ANG II by removing His-Leu residues from the C-terminal end [14]. 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 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 a vasodilator angiotensin (1-7) thus counters the activity of the ACE by reducing the amount of ANG-II and increasing ANG (1-7) which subsequently binds to Mas receptor (MAS R) causing vasodilation [15]. This cascade of events hence is referred to as vasoprotective arm of RAAS (Fig. 1). Notably, ACE also degrades bradykinin, a potent vasodilator and other vasoactive peptides [16, 17]. Thus, the same ACE that generates a vasoconstrictor (ANG II) also disposes of vasodilators (bradykinin). On the other hand, ACE2 degrades does not degrade bradykinin [18].

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 (Figure 1).

Figure 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 SARS-CoV-2 infection

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 [19–21]. It triggers imbalanced T cell activation against the virus hence a massive inflammatory cascade

termed 'cytokine storm' is ensued eventually [23]. This spurring immune response along with attacking the virus, leads to the pulmonary cell destruction [24, 25]. Consequently, 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 (+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 [26, 27].

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 [19–22]. The ACE2 receptor, a trans-membrane type I glycoprotein, was initially discovered by two independent groups in year 2000 and has a 40% structural identity to ACE [28, 29]. This monocarboxypeptidase has 805 amino acids with one extracellular catalytic domain that catalyzes removal of one amino acid from C- terminal end of ANG II and convert it to ANG (1-7).

While ACE2 serves as the receptor for SARS-CoV-2 binding and subsequently entering host cells [20], 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 [30], hence is not unlikely to see a reduced expression of ACE-2 after SARS-CoV-2 infection.

In vivo experiment involving a rat model of acute respiratory distress syndrome has shown to increase ACE activity and ANG II expression, but reduce ACE2 activity and ANG (1–7) levels [31, 32]. Furthermore, a negative correlation was shown between ACE2 expression and COVID-19 fatality at molecular levels [33]. Again, SARS-CoV-2 genome was found in 7 heart samples, characterized by increased myocardial fibrosis, inflammation, and reduced myocardial ACE2 expression while studying post-mortem autopsy of heart tissues from 20 COVID-19 patients [34].

At the same time, patients with cardiovascular diseases have increased ACE2 as compared to healthy controls [8]. 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 [35], 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 ACE 2 as an epicentre of the pathogenesis (Fig 2).

Figure 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]

Therefore, COVID-19 patients are assumed to face higher cardiovascular damage during disease progression [36]. This can be reasonably argued that as 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 is consequently ensued.

Impact of CVD treatment in COVID-19 comorbidity

A range of medications are prescribed for the 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 abnormally activated RAAS system responsible for elevated blood pressure in human body.

If this RAAS gets abnormally activated, the balance gets violated resulting in continuous vasoconstriction and hypertension. As a method of restoration, medications like ACE inhibitor are used to relax blood vessels by blocking the formation of ANG II that narrows blood vessels [37] while ARB helps relax blood vessels by blocking the action of ANG II on its receptors [38]. Both ACE inhibitors and ARBs can reduce angiotensin II levels [38]. In general, ACE inhibitors reduces 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 rennin hormone from kidney that starts the RAAS [39].

Antihypertensive agent used to treat high blood-pressure patients including ARBs, ACE inhibitors and RAAS inhibitors can upregulate ACE2 expression in rodent studies, hence are suspected to potentially increase viral entry sites for coronaviruses worsening the outcome in patients with COVID-19 [40].

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 significant increase of ACE2 protein expression in rats with acute lung injury [41]. While, ACE2 has been reported to have protective effects in acute lung injury by Imai et al [43]. Contrarily, other ACE inhibitor (lisinopril) and ARB (losartan) alone or in combination did not increase cardiac ACE2 activity but caused 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

[42]. Therapeutic intervention with cyclic form of ANG-(1-7) attenuated the inflammatory mediator response, markedly decreased lung injury scores, and increased oxygenation [32].

The above-mentioned evidences demonstrate that therapeutic interventions for CVD such as 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 SARS-CoV-2 to enter host cells resulting in fatal consequences for COVID-19 patients, or (2) improving cardiovascular conditions in case functional expression of ACE2 is reduced due to SARS-CoV-2 infection. The later 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, 43].

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

Notably, ACE2 is greatly expressed in epithelial cells of alveoli, trachea, bronchi, bronchial serous glands [21], and alveolar monocytes and macrophages, as well as in coronary vessels along with cardiac myocytes and fibroblasts [44].

Potential therapeutic targets for COVID-19 patients with CVD

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 replication.

First of all, 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 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 [18] (Fig. 3, indicated with 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 letter b). 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 letter c).

Figure 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/reduction]

Beneficial roles of Zinc supplement in the potential therapeutic interventions

Zn²⁺ is stable in a biological environment such as cell cytoplasm hence serves as an ideal metal cofactor for many biological reactions. As a Zn-metalloenzyme, functional expression of ACE2 [18] 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 polymerase (RdRp) activity by inhibiting the SARS-CoV RdRp elongation and template binding [45].

Earlier it was also shown that Zn²⁺ inhibited the proteolytic processing of replicase polyproteins [46, 47]. 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 [48], pyrithione [45, 49], zincophorin [50], and hydroxychloroquine [45, 51, 52]. 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 [53].

In addition to those direct effect on the virus as well as to improve clinical outcome of CVD treatment, a number of immunome pathways such as NF-κB signaling pathway are activated by Zn²⁺ [54]. 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 [55, 56].

Zinc administration in mixed lymphocyte cultures was shown to induce and stabilize a subset of CD4⁺ T cells while both CD4⁺ and CD8⁺ T cells are critical in antiviral immunity [57, 58]. The ability of Zn²⁺ to inhibit 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 [59], respiratory syncytial virus [60], and several picornaviruses [52, 61, 62]. Their interference with polyprotein processing in cells infected with human rhinovirus and coxsackievirus B3 is well evidenced [52].

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 [45, 63, 64]. Zn but not Mg salts namely Zn-sulfate and Zn-acetate were shown to inhibit viral sense and antisense RNA levels by approximately 50%, thus inhibiting viral replication [65].

Conclusion

COVID positive patients' cardiac health, with or without respiratory symptoms could be evaluated for resolving primary complication of COVID-19 and to reduce mortality from potential cardiovascular conditions. Based on the patients' condition, selective treatment plan might be required to ensure minimal lung injury or aggravating the cardiovascular pathologies. Special attention might be required to influence on the functional expression of ACE2. Furthermore, Zn²⁺ supplement with an appropriate ionophore in combination 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 [66, 67], and (3) restoring functional balance of ACE2.

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