Randomised controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol

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ABSTRACT

Introduction SARS-CoV-2 (COVID-19) has caused an international pandemic of respiratory illness, resulting in significant healthcare and economic turmoil. To date, no robust vaccine or treatment has been identified. Elemental zinc has previously been demonstrated to have beneficial effects on coronaviruses and other viral respiratory infections due to its effect on RNA polymerase. Additionally, zinc has well-demonstrated protective effects against hypoxic injury—a clear mechanism of end-organ injury in respiratory distress syndrome. We aimed to assess the effect of high-dose intravenous zinc (HDIVZn) on SARS-CoV-2 infection. The end of study analyses will evaluate the reduction of impact of oxygen saturations or requirement of oxygen supplementation.

Methods and analysis We designed a double-blind randomised controlled trial of daily HDIVZn (0.5 mg/kg) versus placebo. Primary outcome measures are lowest oxygen saturation (or greatest level of supplemental oxygenation) for non-ventilated patients and worst PAO2/ FiO2 for ventilated patients. Following power calculations, 60 hospitalised patients and 100 ventilated patients will be recruited to demonstrate a 20% difference. The duration of follow-up is up to the point of discharge.

Ethics and dissemination Ethical approval was obtained through the independent Human Research Ethics Committee. Participant recruitment will commence in May 2020. Results will be published in peer-reviewed medical journals.

Trial registration number ACTRN12620000454976.

INTRODUCTION

Since December 2019, a new coronavirus, designated SARS-CoV-2, has caused an international pandemic of respiratory illness termed COVID-19, posing significant threats to global health and the economy. In more severe cases, COVID-19 enters the lungs, causing respiratory complications such as bronchitis and pneumonia. Development of pneumonia leads to a reduced ability for oxygenation and in some cases, the development of acute respiratory distress syndrome, requiring mechanical ventilation. In the most severe cases, patients can develop multiple organ failure and hypoxic brain injury. In the absence of an effective vaccine or robust treatment for people with the disease, there is an urgent need to find a treatment that inhibits virus replication or reduces the progression of the disease.

Zinc is a naturally occurring essential heavy metal, and zinc deficiency is associated with a range of pathological conditions, including retarded growth and delayed wound healing and tissue repair. Zinc is also important for the maintenance and development of the immune system and plays a role in cell division and growth. Zinc deficiency results in reduced immunity and increases susceptibility to infectious diseases. Numerous studies report the potential of zinc and zinc salts to inhibit viral infections in clinical and experimental settings. Viral infections that have been successfully inhibited by zinc include the common cold (a type
of coronavirus), respiratory syncytial virus infections, cytomegalovirus infections and herpes labialis. More importantly, zinc is a potent inhibitor of the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus in cell culture.

Furthermore, our published studies have shown that high-dose intravenous zinc (HDIVZn) protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia. In addition to being efficacious, the dose of elemental zinc used for this HDIVZn trial (0.25 mg/kg ZnCl₂) and applied in our preclinical study in sheep is very safe and well-tolerated in humans as verified from multiple observational reports including the treatment of very sick children in an intensive care unit (ICU) setting with suspected zinc deficiency.

We plan to perform a single-site randomised controlled trial to test the efficacy and safety of HDIVZn administered as a daily injection in subjects with COVID-19 infection to assess whether giving HDIVZn to patients improves clinical outcomes.

METHODS AND DESIGN

Ethical approval was sought through the Austin Health institutional Human Research Ethics Committee (Version 2, 4 April 2020). Any modifications to the trial protocol will be approved prospectively by the associated Ethical Committee. This clinical trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976). The protocol design was produced in adherence with the Standard Protocol Items for Randomised Trials. No external funding was sought for the completion of this project.

Patient and public involvement

The development of the research question stemmed from the urgent global crisis inflicted by COVID-19, and the urgent need for a therapy to reduce the impact of the disease on the affected patients. Patients were not intrinsically involved in the design, recruitment or conduct of the study. Results of the study will be disseminated to patients after publication in peer-review journal. While the burden of the treatment or placebo was not assessed directly by patients, it was deemed minimal due to the lack of deviation from standard care.

Study design

This is a phase 2, double-blind, placebo-controlled, randomised study at a single institution. The study plans to randomise 160 hospitalised participants, including 100 critically ill patients with confirmed COVID-19 (as detailed in table 1) and to evaluate the efficacy and safety of HDIVZn over a 7-day period of treatment.

Dosing rationale

The upper limit for daily zinc intake in an adult is 40 mg. However, when 30 mg of elemental zinc was given orally to humans, it resulted in an only 1.8-fold increase in plasma zinc in the first 4 hours. A similar study in humans where elemental zinc (30 mg/d) or placebo were given orally for 6 months showed a statistically significant in the plasma zinc from 14.18±1.75 μmol/L in the placebo group to 17.18±3.48 μmol/L in the zinc group. Oral delivery of zinc is affected by several factors, including normal variations in gut zinc absorption, dietary factors such as the presence of phytate, and interactions with other metal ions. Also, repeated high oral zinc intake causes a rapid and significant upregulation of intestinal metallothioneins (MTs) which markedly decrease subsequent gut zinc, and importantly copper, absorption. The latter may lead to copper deficiency in patients administered zinc for prolonged periods.

In our recently published sheep study, we determined that a single IV dose of ZnCl₂ at 0.5 mg/kg increased the plasma zinc concentration by fourfold from a baseline concentration of 11.3±0.4 μmol/L to 46.5±1.5 μmol/L within 2 hours. Interestingly, two doses of IV ZnCl₂ at 0.5 mg/kg increased the plasma zinc concentration sevenfold from a baseline concentration of 11.3±0.4 μmol/L to 70.1±5.8 μmol/L.

Translation of a drug’s dose from animals to humans depends on the drug dose correction factor (Kₘ) which is calculated by dividing the average body weight (kg) of a species by its body surface area (m²). For humans, Kₘ is 37. Direct carryover of a drug’s pharmacologic dosage, from animals to humans depends on how similar the Kₘ value of the animal species in which efficacy was tested, is to human Kₘ. As published by Nair and Jacob, Kₘ for rats is 6, for rabbits is 12 and for sheep is 36 (average body weight of sheep is 40 kg, and body surface area is 1.10 m²). Therefore, the near similar Kₘ value of sheep (36) to that of humans (37) has allowed us to conclude that human equivalent elemental zinc dosage would equate to 0.25 mg/kg, the same dose that was shown to be protective in the sheep study and which we plan to use in our proposed trial.

Information regarding the safety of HDIVZn can be derived from published reports where humans were treated with high doses of zinc. The estimated elemental HDIVZn dosage for an average 70 kg human to be used in our proposed study will be 0.25 mg/d x 70kg=17 mg/d. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5 mg/d for 8 successive days) in the treatment of human burns without any side effects. In fact, zinc at doses ranging from 5 to 22 mg/d has been administered in humans routinely as a component of parenteral nutrition without any reported side effects. Furthermore, a recently published phase I clinical trial in critically ill children with suspected zinc deficiency involved administration of zinc intravenously at a dose of three times higher than is proposed in the current study (0.75 mg/kg/d for 7 days), without any adverse effects.

The duration of daily dosing in the current trial was based on previous research assessing dose escalation of
intravenous supplemental zinc.20 This dose escalation study in paediatric critical illness highlighted that with sufficiently high doses of intravenous zinc, a treatment course of 7 days resulted in increases of serum zinc.

PARTICIPANTS AND RANDOMISATION

Eligibility

Patients will be eligible for randomisation in this study if they are consenting symptomatic hospitalised adult patients fulfilling WHO’s case definition for COVID-19, including a positive PCR for COVID-19 from any specimen (eg, respiratory, blood, urine, stool, other bodily fluid) within 14 days of presentation. Broadly speaking, patients included will be those with respiratory associated COVID-19 disease. Patients with alternate acute respiratory pathology causing respiratory compromise, such as bacterial pneumonia, will not be deemed suitable for randomisation. Inclusion and exclusion criteria are summarised in table 1. Patients enrolled in the current trial are eligible to be recruited in alternative trials and may receive other therapeutic interventions including dexamethasone or remdesivir.

Screening procedures

The screening log is designed to monitor patient recruitment. A screening log will be maintained at the participating site by the research coordinator to document patients evaluated for enrolment. The log will provide a record of all patients assessed for eligibility and deemed ineligible for the study. When a patient is considered ineligible, the reason(s) will be noted on the log. The log will also be used to assess patient recruitment targets. Patients will be consented by trial investigators by completion of consent and assent forms from the patient directly or surrogates if the patient does not have the ability to consent (online supplemental 1).

Randomisation

This study will be conducted as a randomised double-blinded placebo-controlled study using a stratified randomisation method. Ward and ICU patients will be enrolled as soon as possible after fulfilling the criteria of stratification. Consenting patients will be stratified based on whether they require mechanical ventilation. Thereafter, a permuted block randomisation method with variable block sizes of 2, 4 and 6 will be used to allocate eligible patients to either the treatment group, receiving HDIVZn
or to the control group in a 1:1 ratio. Randomisation will be performed by the randomisation module in Research Electronic Data Capture (REDCap, Vanderbilt University, USA), which is a secure web application for managing online data collection. Patients, clinical staff, outcome assessors and data analysts will be blinded. Assessment of the unblinding procedures by the project research officer in the case of adverse event will be performed if necessary. The clinical staff involved in patient care will administer the trial drugs as soon as possible after enrolment.

**Study drug administration and blinding**

Pharmaceutical grade zinc chloride stock solution obtained from an Australian company (Phebra Pty Ltd, Lane Cove West, NSW, Australia) will be diluted in 250 mL of normal saline and infused, resulting in a final dosage of 0.5 mg/kg/d. Patients will be administered zinc daily for 7 days. Placebo will be unadjusted 250 mL of normal saline. To standardise administration time, zinc infusions or control will commence in the early morning. Zinc or placebo will be administered via central venous or peripheral access over 3 hours. Zinc or placebo will be administered daily until either discharge or 7 days of infusion (depending on which is sooner). The clinical trial nurse (allocation concealment) will use a web-based randomisation programme to determine the allocation of patients and then prepare the coded zinc solution or placebo. Each coded solution bag, which will be indistinguishable irrespective of study group, will then be dispensed for administration to the patient as per protocol. This coded identifying study number will also be labelled on the patient case report form (CRF). The investigators, study coordinators, treating physicians, bedside nurses and patients/family will remain blinded to the allocated study solution.

**STUDY ASSESSMENT**

**Study objective and endpoints**

The endpoints of this study are listed in table 2. The primary objective is to assess the effect of 7 days of HDIVZn on oxygenation in comparison with placebo in patients with confirmed COVID-19. Specifically, oxygen saturations and the requirement of supplemental oxygenation (litres/min) to maintain acceptable saturations will be measured at various time points in conjunction with measurement of routine observations. If applicable, the method of supplemental oxygenation provided will be recorded (eg, nasal prongs, Hudson mask). The primary outcome will be measured by either the highest level of supplemental oxygen (non-ventilated patients) or lowest PaO$_2$/FiO$_2$ ratio in ventilated patients.

Secondary outcome measures include ICU and in-hospital mortality, length of stay in ICU or hospital, duration of supplemental oxygen, severe adverse drug events and changes based on WHO R&D Blueprint ‘Novel Coronavirus COVID-19 Therapeutic Trial synopsis’. The following is based on an eight-point ordinal scale consisting of:

- 0, not hospitalised, no clinical or virological evidence of infection;
- 1, not hospitalised, infected and able to resume normal activities;
- 2, not hospitalised, infected, but unable to resume normal activities;
- 3, hospitalised, no requirement of supplemental oxygen;
- 4, hospitalised, requiring oxygen therapy via mask or nasal prongs;
- 5, hospitalised, non-invasive ventilation, requiring high flow oxygen;
- 6, hospitalised, intubation and mechanical ventilation;
- 7, hospitalised, requiring Extracorporeal membrane oxygenation (ECMO), invasive mechanical ventilation, additional organ support, Renal replacement therapy (RRT);
- 8, death.

**Physical examination, measurements and laboratory tests**

All data will be collected by blinded trained staff using a CRF worksheet developed by the study team. Data will then be entered into the REDCap web database (electronic CRF). Randomised patients will be followed up to discharge, death or 28 days postrandomisation whichever occurs first.
Study day 1 commences on randomisation and concludes at the expiry of the calendar day. Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events. A complete list of collected data is summarised in Table 3. Compliance of study protocol will be monitored by daily checklists confirming administration of trial infusion and collection of laboratory investigation and minimum outcome measures.

Daily patient assessment will be performed to encourage participant retention, completion of infusion and data collection. Further, daily assessment will allow for prompt recognition of adverse effects of the trial intervention.

### Adverse events

An adverse reaction is defined as any untoward and unintended response to an investigational medicinal product

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**Table 3** Collected data during trial

| All patients | Non-ventilated patients | Ventilated patients |
|--------------|-------------------------|---------------------|
| **Baseline data** | | |
| Demographics: age, gender, place of residence | Daily saturations (worst values) | Daily PaO2/FiO2 ratio |
| Comorbidities: diabetes mellitus, arterial hypertension, congestive heart failure, chronic obstructive airways disease, chronic liver disease, malignancy, chronic renal failure | Daily oxygen flow | Daily oxygen flow |
| Preadmission medication | Fluid input | Fluid input |
| Functional status/frailty score | Urine output | Urine output |
| **Daily observations** | Fluid balance | Fluid balance |
| | Daily lactate level | Vasopressor data |
| | | |
| **Laboratory investigations (collected at 0800)** | | |
| Daily serum creatinine | Daily liver function | Daily serum creatinine |
| Daily liver function | Daily blood count | Daily liver function |
| Daily blood count | Daily zinc and trace metal concentration (copper, potassium, magnesium) | Daily blood count |
| Daily zinc and trace metal concentration (copper, potassium, magnesium) | Daily cardiac troponin | Daily zinc and trace metal concentration (copper, potassium and magnesium) |
| Daily cardiac troponin | Daily lactate level | Daily cardiac troponin |
| Daily lactate level | | Daily lactate level |
| | | |
| **Primary outcome measures** | | |
| Mean change in highest level of oxygenation requirement (oxygen flow in litres/min) | Mean change in lowest PaO2/FiO2 ratio (in mm Hg) |
| | | |
| **Secondary outcome measures** | | |
| Mortality (time frame: up to day 28) | Mortality (time frame: up to day 28) |
| Duration of oxygen therapy (days) | Duration of mechanical ventilation (days) |
| Duration of hospitalisation (days) | Duration of oxygen therapy (days) |
| Length of stay in the intensive care unit (ICU) and hospital | Duration of hospitalisation (days) |
| Frequency of serious adverse drug events | Length of stay in the ICU and hospital |
| Acute kidney injury | Frequency of serious adverse drug events |
| Acute liver injury | Acute kidney injury |
| Time to resolution of fever for at least 48 hours without antipyretics by clinical severity | Acute liver injury |
| Incidence of severe or life-threatening bacterial, invasive fungal or opportunistic infection | Use, duration and dosage of vasopressor drugs |
| Number of patients admitted into an ICU (time frame: up to day 28) | Time to resolution of fever for at least 48 hours without antipyretics by clinical severity |
| Sequential organ failure assessment (SOFA) respiratory score (time frame: 28 days). Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) | Incidence of severe or life-threatening bacterial, invasive fungal or opportunistic infection |
| Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint ‘Novel Coronavirus COVID-19 Therapeutic Trial Synopsis’. | Number of patients admitted into an ICU (time frame: up to day 28) |
| – Percentage of patients reporting each severity rating on an eight-point ordinal scale (time frame: day 14) | SOFA respiratory score (time frame: 28 days). Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) |
| – Time to improvement in one category from admission using the eight-point ordinal scale (time frame: up to day 28) | Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint ‘Novel Coronavirus COVID-19 Therapeutic Trial Synopsis’. |
| – Mean change in the eight-point ordinal scale (time frame: up to day 28) | – Percentage of patients reporting each severity rating on an eight-point ordinal scale (time frame: day 14) |
| – Time to improvement in one category from admission using the eight-point ordinal scale (time frame: up to day 28) | – Time to improvement in one category from admission using the eight-point ordinal scale (time frame: up to day 28) |
| – Mean change in the eight-point ordinal scale (time frame: up to day 28) | – Mean change in the eight-point ordinal scale (time frame: up to day 28) |
related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product will qualify as adverse reactions. Adverse events were classified per the Common Terminology Criteria for Adverse Events (CTCAE V.5). All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator’s judgement will be reported. An interim analysis is planned after 50% (80) of the patients have been randomised. Development of severe adverse events (grade 4), such as severe kidney or hepatic injury, will result in cessation of the trial for the participant and all infusions related to the trial will be stopped. For patients suffering adverse events, aftercare will be provided by in-hospital specialists units as part of ongoing care. Additionally, the treatment will be discontinued in cases where the participant wishes to withdraw from the trial.

Safety evaluations
An independent Data Monitoring Committee (DMC), consisting of experts in intensive care, clinical research and biostatistics will be established before patient enrolment and will review all trial protocols. The role of the DMC will be to provide study oversight to ensure that the rights and safety of patients involved in the study are protected by reviewing reported Adverse Events and making recommendations to the Management Committee.

STUDY ANALYSIS
Sample size
The primary outcome of this study is related to the effect of zinc therapy on the level of oxygenation expressed either as worst (highest) oxygen flow (in litres/min) in non-ventilated patients or worst (lowest) PaO2 (in mm Hg)/FiO2 (as a fraction of 1) ratio in ventilated patients. We hypothesise that zinc therapy will decrease the worst level of oxygenation during the 7 days (of the treatment period) by 20% compared with placebo from a mean worst value of 10 L/min (placebo) to a mean of 8 L/min (zinc) or for PaO2/FiO2 ratio from a mean worst value of 150 (placebo) to a mean worst value of 180 (zinc). If patients transition from non-ventilated to ventilated during the study period, the PaO2/FiO2 ratio will be used.

To have an 80% power to see such an effect at an alpha of 0.05 in non-ventilated patients, assuming a SD of the variable, distribution of residuals, ease of interpretation and impact on main results. The coprimary outcome analyses will compare (1) the change in highest level of supplemental oxygenation in non-ventilated patients, and (2) the change in lowest PaO2/FiO2 ratio in ventilated patients through the use of multivariable linear regression. Secondary outcome analyses will involve a mix of linear, logistic and ordinal logistic regression modeling. Assumptions of the relevant models will be checked along with alternative transformations or non-parametric methods as appropriate.

Tests of interaction between dichotomised variables and treatment pathway will be carried out to test for differing treatment effect between participants. Sensitivity analyses will be conducted to explore the robustness of the estimate of the effect. Analyses will include complete case analysis; per-protocol analysis, adjustment for baseline and adjustment for imbalance at baseline (if baseline measures differ substantially).

A p-value 0.05 will indicate statistical significance. A full model with clinical relevant covariates (eg, sex, age, previous heart surgery, preoperative creatinine) will be used for a stepwise backward variable selection procedure to identify independent risk factors for acute kidney injury. A senior statistician will perform data analysis on an intention-to-treat basis. An interim analysis on the safety and the primary outcomes will be performed when 50% (80/160) of the patients have received zinc or placebo for at least 7 days. Summary statistics will be used to describe the clinical data and presented as mean±SD, median with IQR or percentages as appropriate. Chi-squared analysis with Fisher’s exact test (when appropriate) and Student’s t-test (Mann Whitney U test for non-normal distributions) will be used to compare data between the active treatment group and the control group with statistical significance declared for probability values of 0.05 or less. Analysis of the outcome of excluded patients due to other trials will be in accordance with the Consolidated Standards of Reporting Trials guidelines. Statistical coding will be made on request.
Reactive oxygen and nitrogen species and zinc have been shown to induce MT mRNA and protein expression. Our published studies have shown that HDIVZn protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia. It should be noted that hypoxia and oxidative stress, result in an increase in reactive oxygen species (ROS), including superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (·OH)—which result in intracellular damage. Zinc appears to limit ROS production by several mechanisms. First, MTs, small cysteine-rich and heavy metal-binding proteins, participate in the intracellular defence against reactive oxygen and nitrogen species and zinc has been shown to induce MT mRNA and protein expression. Second, zinc competes with Fe$^{2+}$ and Cu$^{2+}$ ions for binding to cell membranes and proteins—normally, these active metals catalyse the production of hydroxyl radical from H$_2$O$_2$ via Fenton chemistry. Third, zinc upregulates the production and activation of antioxidant proteins, molecules and enzymes such as glutathione, catalase and superoxide dismutase, which catalyse O$_2^-$ to oxygen or H$_2$O$_2$. Finally, zinc reduces the activation of oxidant-promoting enzymes such as inducible nitric acid synthase and NADPH enzyme, which catalyse oxygen to O$_2^-$. Accordingly, we hypothesise that Zinc may provide protection against the hypoxic injury that critically ill patients with COVID-19 may experience.

The specific strengths of the current protocol design are (a) prolonged exposure of HDIVZn and (b) assessment of critically ill patients, a population where a benefit would be observed if truly present. There is an inherent difficulty in assessing pulmonary response in clinical trials, and accordingly, the primary outcome measure is a possible criterion. However, maximal oxygen requirements and PaO$_2$/FiO$_2$ are well established surrogate markers and have been used in recent comparable trials.

The safety of HDIVZn has been addressed in previous literature. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5 mg/d for 8 successive days) in the treatment of burns and did not produce any side effects in humans. In the setting of critically ill patients, zinc intravenously at three times higher dose than the current study (0.75 mg/kg/d for 7 days) did not produce any adverse effects.

In conclusion, we designed a single-centre, double-blind, randomised controlled trial to assess the potential benefit of HDIVZn for hospitalised or critically ill patients with SARS-CoV-2 infection and associated respiratory compromise. We believe that our well-designed trial will be able to expediently identify a potential agent that may improve outcomes for these critically ill patients.

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**Contributors** MP assisted in protocol design, ethical submission and manuscript production. LQ and VC were involved in data collection and manuscript production. LJ was involved in statistical design and analysis. PJ, JT and CFMD were involved in patient care and study protocol design. DJ, DB, RB, OP and JI were involved in project design, patient care, manuscript production and supervision.

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**DISCUSSION**

This single-centre, double-blind, randomised controlled trial will assess the role of HDIVZn in protection against the sequelae associated with SARS-CoV-2 virus. The beneficial effects of zinc on viral infections have been previously demonstrated. Further, HDIVZn may provide additional protection to other end organs that may be indirectly affected by pulmonary injury and impaired oxygenation.

In coronaviruses, zinc inhibits both the proteolytic processing of replicase polyproteins and the RNA-dependent RNA polymerase (RdRp) activity. Although mechanisms of action of zinc are unknown, several possibilities exist. First, DNA and RNA polymerases use divalent metal ions like Mg$^{2+}$ as a cofactor, and one possible mechanism is that zinc displaces Mg$^{2+}$ and subsequently inhibits RdRp activity. In support is the observation that various divalent metals ions sustained the activity of poliovirus RdRp in the following preference Mn$^{2+}$ > Co$^{2+}$ > Ni$^{2+}$ > Fe$^{2+}$ > Mg$^{2+}$ > Ca$^{2+}$ > Cu$^{2+}$. In contrast, zinc was incapable of sustaining RdRp catalysed nucleotide incorporation. Second, a zinc-binding pocket has been identified in the Dengue virus and SARS-coronavirus RdRp. Therefore, it is possible that binding of zinc may induce a structural change in the conformation of RdRp which disables RdRp to catalyse nucleotide incorporation. Finally, adding high concentrations of zinc ions to cells impairs viral polyprotein processing which is integral to virus replication.

In addition to the direct effect on viral replication and activity, HDIVZn may play a protective role in alternate organs. Zinc has been demonstrated to be beneficial in reducing mortality in patients with severe pneumonia. Further, in SARS-CoV-2, respiratory compromise results in impaired oxygenation and hypoxia to various end organs. Such hypoxia may contribute to end-organ failure and increase the risk of mortality. Specifically, such COVID-19 associated hypoxia has been proposed to be contributory to cardiac injury, hepatic injury and renal injury. Our published studies have shown that HDIVZn protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia. It should be noted that hypoxia and oxidative stress, result in an increase in reactive oxygen species (ROS), including superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (·OH)—which result in intracellular damage.
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