An overview of SARS-CoV-2 (COVID-19) Infection and the Importance of Molecular Hydrogen as an Adjunctive Therapy.

Authors: Grace Russell[1], Mubasher Rehman[2], Tyler W. LeBaron[3,4], David Veal[1], Emmanuel Adukwu[1], John T Hancock[1*]

1. Department of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol, England.
2. Department of Microbiology, Hazara University Mansehra, Pakistan.
3. Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic.
4. Molecular Hydrogen Institute, Enoch, Utah, USA

*Corresponding author:
John T. Hancock
Faculty of Health and Applied Sciences
UWE,
Coldharbour Lane,
Bristol, BS16 1QY, UK
Email: john.hancock@uwe.ac.uk

Short Title: Hydrogen treatment for COVID-19

Abbreviations:

ACE2 angiotensin-2 converting enzyme**, ARDS** acute respiratory distress syndrome**, ATP** adenosine triphosphate**, BALF** bronchial alveolar lavage fluid, **CAT** catalase, **COPD** chronic obstructive pulmonary disorder**, COVID** corona viridae infectious disease, **DUOX** dual oxidase, **GAPDH** glyceraldehyde-3-phosphate dehydrogenase **GM-CSF** granular macrophage colony stimulating factor, **GSH** glutathione, **GST** glutathione S transferase, **HBV** Hepatitis B virus, **HRW** hydrogen-rich water, **IF** interferon, **IL** interleukin, **MDA** malondialdehyde, **MAPK** mitogen-activated protein kinase, **MERS** Middle-Eastern respiratory syndrome**, MPO** myeloperoxidase, **MODS** multiple organ dysfunction syndrome, **NADH** nicotinamide adenine dinucleotide, **NADPH** nicotinamide adenine dinucleotide phosphate, **NFκB** nuclear factor kappa-light-chain-enhancer of activated B cells, **NRF2** Nuclear factor erythroid 2-related factor 2, **NOX** NADPH oxidase, **ORF** open reading frame, **OS** oxidative stress, **ROS** reactive oxygen species, **RNS** reactive nitrogen species, **SARS** severe acute

respiratory syndrome, **SOD** superoxide dismutase, **T2DM** type 2 diabetes mellitus, **TNFα** tumor necrosis factor alpha, **WHO** World Health Organization, **XOD** xanthine oxidase

Keywords: COVID-19; SARS-CoV-2; Cytokine Storm; Molecular Hydrogen; Reactive Oxygen Species; Virus

Abstract

SARS-CoV-2 is an emerging β-coronavirus that causes COVID-19 disease that manifests primarily as a pulmonary infection that can rapidly progress into severe and acute respiratory distress in susceptible patients. Initial reports of severe pulmonary infections first arose in December 2019 and were reported to the World Health Organization by the Wuhan Municipal Health Commission, China. Within months SARS-CoV-2 rapidly disseminated across the globe causing an unprecedented pandemic that has reached every inhabited continent and provoked an international response into research involving multiple disciplines to combat this novel contagion. Molecular hydrogen (H_2) has shown potential as an emerging and effective therapy for numerous diseases, particularly those which involve excessive production of inflammatory agents as well as reactive oxygen/nitrogen species. Of pertinence to the investigations of SARS-CoV-2 infection are the increasing reports that suggest that H_2 has therapeutic qualities in the treatment of chronic inflammatory lung conditions, and as such it is likely that this diatomic gas may alleviate the severe pulmonary symptoms of COVID-19. Here, the aim is to review the current research into SARS-CoV-2 and to better understand how treatment with molecular hydrogen is likely to affect cellular responses during SARS-CoV-2 infection.

Introduction

H_2 is an uncharged, non-polar, diatomic molecule with a low molecular weight of 2.016 g/mol. These characteristics make H_2 highly favorable for medicinal use as they allow H_2 to diffuse through both the membrane of the cell and those that occur around organelles, such as mitochondria [1, 2]. The distribution of H_2 across the membranes is not affected by electrochemical gradients and it can pass through the hydrophobic phase of lipid bilayers. This allows molecular hydrogen to influence cytosolic reactions and organelle biochemistry. These fundamental properties make H_2 an ideal therapeutic for targeting dysfunctional intracellular processes including metabolic regulation and redox homeostasis. Both of these biological factors that have been reported to contribute greatly to the pathological progression of both infectious and non-infectious diseases [3].

Since the beginning of the 21st century, coronaviruses have caused two major global epidemics. In 2003, with a 10% lethal rate, severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) emerged as a unique and original human-contractable virus, which disseminated globally, reaching the five densely populated continents of Africa, Asia, Europe and both the North and South Americas [4]. Additionally, in 2012 another contemporary and zoonotic coronavirus emerged in Saudi Arabia, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), a potentially fatal infection that also proliferated to other countries across the world, and which has a mortality rate of 35% [5].

Coronavirus infectious disease 2019 (COVID-19) is caused by the SARS-CoV-2 virus, and it is an emerging and novel respiratory disease. SARS-CoV-2 is new strain of human-to-human transmissible coronavirus comprising of positive-sense, single-stranded RNA. The disease initially emerged in the city of Wuhan, Hubei province, People's Republic of China, in late 2019. The Chinese government initially reported the outbreak on 31st December 2019, and within the month the World Health Organisation (WHO) declared a public emergency (30th January 2020). Less than 6 weeks later (11th March 2020) the WHO announced that COVID-19 had reached global pandemic status. This rapid

spread is due, at least in part, to the virus having a predicted basic transmission rate of >2.2 on discovery [6].

COVID-19 is known to present with variable symptoms that range from asymptomatic, yet contagious, to obvious symptoms of respiratory infection with patients regularly presenting with a dry and persistent cough accompanied by a fever in excess of 37.8^{O}C. In some individuals these symptoms can rapidly progress to respiratory failure due to alveola damage that necessitates assistance from a mechanical breathing apparatus. COVID-19 can also lead to excessive production of inflammatory biochemicals, which is referred to as a cytokine storm. It is proposed that H_2 therapy attenuates the damage to alveola cells by remediation of destructive reactive oxygen/nitrogen species (ROS/RNS) and through the reduction of excessive inflammation [7]. Undoubtedly one of the primary functions of H_2 utilization in a clinical setting are the selective ROS antioxidant properties, first demonstrated by Ohsawa *et al.* (2007) in a rodent model of ischemia/reperfusion injury [8]. Their results describe a selective reduction of deleterious hydroxyl radicals ($^{\bullet}OH$) and peroxynitrite ($ONOO^{-}$) molecules, but no reduction in the important signaling molecules hydrogen peroxide (H_2O_2) and nitric oxide ($NO^{\cdot}$) [8]. Correspondingly, it is proposed that due the ability to diffuse through biological membranes coupled with both anti-inflammatory and selective antioxidant properties, H_2 may dampen the effects of the damaging cytokine storm [9], that underlies acute and severe SARS-CoV-2 symptomology.

Currently, however, treatment strategies vary between countries, and a consensus on a globally accepted and definitive treatment plan has yet to be agreed upon. Nevertheless, The National Health Commission of the People's Republic of China have provided a detailed intervention strategy that includes administration of various antiviral medicaments (e.g. lopinavir, ritonavir, chloroquine phosphate) along with inhalation of molecular hydrogen (66:33%; H_2:O_2) as an adjunctive therapeutic that is able to be delivered through nasal cannulas [10].

Aims

This review aims to assess the current research into COVID-19, a novel corona virus that has caused a global pandemic, and to better understand how treatment with molecular hydrogen may affect cellular responses during severe SARS-CoV-2 infection.

COVID-19: 'Halo' to a New Contagion

SARS-CoV-2 (GenBank Accession: MN908947), colloquially referred to as COVID-19 virus, is part of SARS related coronavirus species that emerged in the Chinese province of Wuhan, December 2019, and as such has only recently been taxonomically classified, as detailed in Table 1. Initially the WHO named the new coronavirus "2019-nCoV" on January 12, 2020 [11]. However, by February 11, 2020, the name Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) replaced 2019-nCoV, and the International Virus Classification Commission, in part due to the high homology with SARS-CoV-1, and the disease caused by SARS-CoV-2 infection was designated as COVID-19 [12].

Intense and rapid study of this new contagion has revealed SARS-CoV-2 to be an enveloped virus with an outer adhesive coating of glycoproteins that form a corona, or halo shape when visualized using transmission electron microscopy (Figure 1). Individual corona viruses are currently categorized into 4 classes, α, β, γ and δ. Contemporary reports suggest that α and β strains infect mammalian hosts, while γ and δ are currently thought to only infect avian species [13]. Outbreaks of such viruses as SARS (GenBank Accession: AY278488.2) (2003/4) and MERS (GenBank Accession: NC_019843.3) (2015), have demonstrated that in particular β-coronaviruses have the ability to transfer between species [14]. To date, only six coronaviruses are known to cause disease in humans, four (α-HCoV-229E, α-HCoV-NL63, β-CoV-HKU1 and β-HCoV-OC43) are known to cause typically surmountable and mild respiratory infections comparable to the common cold [15]. The other two viruses, SARS-CoV-1 and MERS-CoV (with zoonotic transmission originating through bats and camels, respectively)

are known to cause severe respiratory distress that may develop or exacerbate multiple co-morbidities including cardiovascular, hepatic, and kidney injuries resulting in multi-organ failure and fatality [16].

Transmission, Incubation and Symptomology

In line with many respiratory virus transmission patterns, SARS-CoV-2 is passed directly through aerosol droplets that are typically inhaled or that reach the soft tissues of the eyes, mouth, or nasal cavity. Infection can also occur through indirect contact with the virion where transmission can be transferred from solid surfaces, such as door handles and bannisters, with evidence suggesting that the virus can survive and remain viable for up to three days (e.g. on steel, plastic, and cardboard) [17]. SARS-CoV-2 is a virulent and unique pathogen and as such, it is yet to be well understood. Consequently, early and accurate diagnosis is critical to understanding the epidemiological status of viral transmission, whilst expeditious and effective treatment strategies are vital to the recovery of acutely affected patients.

A study by Wang *et al.,* noted that the incubation period of COVID-19 ranged from 2-14 days [18]. Whilst a further study by Laur and associates [19] elucidated that the median incubation period was estimated to be 5.1 days (95% CI, 4.5 to 5.8 days), with 97.5% of those developing symptoms within 11.5 days (CI, 8.2 to 15.6 days). Alarmingly, the range between showing symptoms to fatality has been determined to be 6-41 days, with an average of 14 days [19]. However, it should be noted that these figures are highly dependent on individual variation including the age and ethnicity of the patient as well as their immune system status. To illustrate, the period between the onset of symptoms and severe symptomology is shorter among the patients >70 years of age [7]. In other individuals, the infection has been shown to present in various forms, ranging from an asymptomatic carrier state to pneumonia and acute respiratory disease. Gastrointestinal symptoms such as diarrhea have also been noted in some COVID-19 patients, a phenomenon also observed in patients infected with SARS-CoV-1 [20]. The range of symptoms currently reported as the knowledge of the disease grows suggests a robust

approach to screening and testing for the virus. For example, urine and fecal sample testing may provide a relatively simple and non-invasive means of antibody or antigen detection, allowing assessment of an individual's infection-status. Also of concern when attempting to control the infection rate of SARS-CoV-2 is that transmission is possible through asymptomatic carriers who are generally unaware that they are infected. This can often result in the enhancement of transmission, especially between vulnerable groups that include the elderly, previously hospitalized patients, and healthcare workers [21].

Common symptoms that have been observed in patients with COVID-19 are associated with the respiratory tract and frequently include a persistent, yet unproductive cough, dyspnoea, fever, and pneumonia that can rapidly progress to severe and acute respiratory syndrome and even death [22]. Comparable to SARS-CoV-1 and MERS, SARS-CoV-2 is a novel β-coronavirus, that causes COVID-19 respiratory disease that can further progress into acute respiratory distress syndrome (ARDS) in a percentage of the population [23], especially the elderly, the immunocompromised, or those with underlying health conditions such as pre-existing respiratory or metabolic disorders [24]. ARDS is a common result of severe pulmonary infection involving Type II cell hyperplasia and membrane permeability, jeopardizing the structural integrity of surfactant-producing alveolar cells [25]. Additional complications in those with severe COVID-19 have been noted to manifest within the cardiovascular and renal systems that can progress into multiple organ dysfunction syndrome (MODS), an immediate and life-threatening condition [12]. Differential identification of COVID-19 may involve assessment of the oxygenation index, myeloperoxidase (MPO) activity, bronchial alveolar lavage fluid (BALF) [26], all of which are parameters that undoubtedly increase the mortality rate for this particular infection.

Epidemiology

Although reported cases of COVID-19 vary between countries, in part due to irregularities in reporting strategies, testing accuracy, and the testing capability of individual countries, as of May 21st 2020, Worldometer [27], data collated from WHO, CDC and NHS reports state the total number of cases in Europe to be 1,843,269 among which 166,089 fatalities have occurred. Additionally, there are 854,065 active cases with 10,891 being deemed as either serious or critical. In North America, the total number of cases is reported to be 1,766,177 with a total of 108,275 deaths occurring. Active cases are recorded as 1,189,424, with 18,977 cases requiring critical care.

In Asia, the total number of cases reported to date is 875,625 among which 26,051 cases were fatal. Active infection rates in Asia equals 343,504 and 4,941 of these are deemed critical cases. The South American countries have totaled 521,321 cases, among these 26,779 fatalities have been attributed to COVID-19 infection. 300,751 active infections have also been described in the South Americas, with 10,583 requiring critical care. In Africa, the total number of cases are 97,905 with 3,022 deaths, 55,260 active infections with only 288 of these viewed to be serious. Oceania total cases had reached 8,688 with 121 deaths, 542 patients with ongoing symptoms, with 10 of these receiving critical care [27]. This equates to an approximate global death rate of 4.62% of confirmed COVID-19 cases with a marked increase noticed in Europe (9.01%) and North America (56.13%), when compared with Africa (3.09%) or Asia (2.97%).

Genomic Identification

Since COVID-19 has been determined as a pandemic strain that has rapidly pervaded throughout the human population, the sequencing of the SARS-CoV-2 genome has been imperative. Originally conducted by researchers in the city of Wuhan, China, December 2019, the first genome sequence was made available to researchers Worldwide, where multiple analysis undertaken in many countries has further confirmed the original sequence [28]. The genome of SARS- CoV-2 is 29.891 kb long and is

reported to have 38% G/C content [16]. As with other documented coronaviruses, the SARS-CoV-2 genome contains 14 open reading frames (ORFs) that encode 27 proteins. ORF1 and ORF2 at the 5′-terminal region of the genome encode for 15 non-structural proteins important for virus replication, whilst the 3′-terminal region of the genome encodes for structural proteins including spike (S), envelope (E), membrane (M), nucleocapsid (N), and eight additional accessory proteins [29]. Of particular interest to the identification of SARS-CoV-2 are the structural glycoproteins responsible for attachment of the virus to the host cell. Interestingly, both SARS-CoV-1 and SARS-CoV-2 both utilize as a receptor the angiotensin-2 converting enzyme (ACE2), a carboxypeptidase located in the airway epithelium and in lung parenchymal tissues [30,31]. The (S) protein of SARS-Cov-2 is comprised of two subunits. The outer S1 subunit initiates adhesion to the host cell whilst the S2 subunit is responsible for virus-cell fusion [31]. Figure 2 details and compares the amino acid sequence of the (S) protein of SARS-CoV-1, SARS-CoV-2, and Bat derived HKU3 coronavirus. Alterations in the respective sequences may begin to explain the different mechanisms of adhesion of these particular viruses, and thus the (S) protein is of great interest as a potential target for medical intervention.

Scrutiny of the SARS-CoV-2 genome reveals a 79% sequence homology with SARS-Cov-1 and a 50% similarity with MERS [16], and whilst SARS-CoV-1 and SARS-CoV-2 utilize ACE2 receptors to gain entry into the body, MERS-CoV shows a preference for dipeptidylpeptidase-4 [32]. This may become a relevant factor when considering suitable pharmacological agents that effectively neutralize viral adhesion and/or replication. Moreover, the comparable sequence of these viruses suggests that the current human-associated coronavirus has mutated from the local bat colonies of China. Wu *et al.*, (2020), however, report that although bats are considered a nutritional delicacy in Chinese culture, and this could provide a possible route of transmission, these products were not available at the wet market thought to be at the center of the current outbreak [33]. This is a cause for concern as protein alignment sequences, alongside phylogenetic analysis [34], show that similar receptor proteins have been described in many species, thus raising the possibility of alternative or intermediate hosts, such as

229 birds, hedgehogs, snakes, turtles or protected pangolins, all of which were noted to have been
230 commercially available in the city of Wuhan [33]. Supporting evidence for the likelihood of bats as
231 the natural hosts of SARS-CoV-2 was published in *Nature*, by Zhou *et al*., who demonstrated a 96.2%
232 sequence similarity with BatCoV RaTG13 (GISAID Accession: EPI_ISL_402131), a virus whose
233 natural hosts are vespertilio, or bat, species [35]. Ongoing research by Shereen and colleagues suggests
234 that SARS-CoV-2 has higher transmission rate when compared with SARS-CoV-1. This has been
235 considered to be a result of genetic recombination of the (S) protein in the RBD region of the SARS-
236 CoV-2 virus, and which is further hypothesized to explain the virus' increased ability to adhere to the
237 human ACE2 receptor [36].
238
239 ## The immune response to COVID-19 and ROS involvement
240 During SARS-CoV-2 infection, as with other respiratory infections, the primary epithelial response is
241 to produce and express physiological distress proteins such as cytokines, chemokines, and growth
242 factors. These include those of the interleukin family whose role it is to direct and recruit leukocytes
243 to the site of infection [37]. Inflammatory mediators also increase the production of ROS within the
244 mitochondria. ROS are also produced by way of upregulated expression of NOX enzymes (e.g. *NOX2*)
245 responsible for generation of these oxidative entities during the respiratory bursts from recruited
246 phagocytes [38]. This can proceed in a cyclical manner, wherein cytokines upregulate ROS activity
247 and elevated ROS leads to an increase in cytokine expression. This is the proposed mechanism by
248 which the extensive and life-threatening parenchymal tissue damage occurs in COVID-19 patients
249 [22], illustrated in Figure 3.
250 Once the cell membrane has been breached the host cell has been demonstrated to respond by
251 activating $CD4^+$ lymphocytes that rapidly differentiate into pathogenic, hyper-inflammatory T-helper
252 cells that express proinflammatory cytokines as tumor necrosis factor alpha (TNF-α) and various
253 growth factors including granulocyte-macrophage colony-stimulating factor (GM-CSF). Of relevance

when considering an increasing oxidative environment during COVID-19 infection is the increase of GM-CSF that particularly induces the expression of $CD14^+$ and $CD18^+$ monocytes known to express the biphasic cytokine interleukin-6 (IL-6) [39].

IL-6 expression becomes significant during COVID-19 infection as this particular cytokine is largely responsible for mediation of the acute-phase immunological response, a defensive physiological reaction involving an increasing body temperature in an attempt to eradicate the pathogen or cause of disease, as well as the activation of inflammatory immune cells, predominantly neutrophils and macrophages [40]. Moreover, inflammatory granulocytes are considered to be a major contributing factor to the increasing oxidative environment by way of the oxidative burst produced by inducible NADPH oxidase (iNOX4), enzymes within the leukocyte allowing the release of copious ROS/RNS species in defense against pathogens. It should also be noted that dedicated enzymes such as nitric oxide synthases (iNOS, nNOS, eNOS) and dual oxidase (DUOX1/2) also contribute to increasing ROS/RNS during cellular stress events [41]. Intense and acute elevation of ROS/RNS not only causes damage to cellular structures via initiation of free-radical cascades, breaching phospholipid membrane integrity, but such an elevation of reactive electrophilic species can also be responsible for the modification of essential metabolic proteins such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). These actions frequently lead to cessation of enzymatic activity [42], and fluctuation or disturbance to the essential energy-producing processes of the cell, which is vital in the body's defense against pathogenic violation.

Current Treatment Strategies

There are no specific antiviral medications or developed vaccines that have been globally recommended for the treatment of COVID-19 [12]. However multiple strategies are being implemented worldwide, many of which include combinations of the drugs listed in Supplementary Table 1. In addition to these antiviral therapies, nosocomial treatment of COVID-19 often involves the requirement of O_2 therapy, typically delivered by nasal cannula for severe respiratory symptoms, or in

critical cases invasive and precarious intubation methods may also be necessary [25]. In such conditions H_2 should be used in combination with other treatments such as Anakinra or Tocilizumab, or in conjunction with O_2, considering H_2 is likely to be a complimentary, restorative molecule. Equally, since H_2 is able to help modulate and regulate cytokines (i.e. production and consequence), it would be fitting to be used in combination with such targeted immunomodulatory drugs.

H_2 – And what it can do

Atomic hydrogen is the first element in the periodic table and the lightest element in the universe. It is a reactive free radical and as such can only exist on Earth in its molecular forms by combining with other atoms, forming a variety of molecules and compounds. When the radical combines with another hydrogen atom, it forms molecular hydrogen, a diatomic gas (H_2) with a molecular weight of 2.016 g/mol [43]. As a result of its low molecular weight, small size, and nonpolar nature, H_2 is highly diffusible and is able to permeate through the blood/brain barrier, lipid membranes, cytosolic fluid, and into the cellular organelles. These properties of H_2 are deemed profoundly favorable considering conventional antioxidants lack these abilities, and are therefore, likely to be therapeutically less effective [44]. The salutary qualities of H_2 were first recognized by Dole and colleagues (1975) who demonstrated that hyperbaric hydrogen therapy, with a dosage of 2.5% O_2: 97.5% H_2 at 8 atmospheres of pressure, can reduce squamous cell carcinoma in murine models [45]. However, it was not until 2007, when Ohsawa *et al*. [8] demonstrated that H_2 had a selective antioxidant effect, favoring reduction of non-signaling ROS/RNS, that the molecular mechanisms of H_2 activity began to be elucidated.

Ongoing research into this area has revealed that H_2 exhibits properties that activate nuclear factor erythroid 2-related factor 2 (Nrf2), a protein known to induce genetic transcription by binding to the antioxidant response element (ARE), a cis-acting enhancer sequence accountable for the consequential expression of >200 cytoprotective peptides, proteins and enzymes [46]. Once released from its partner

protein KEAP1, Nrf2 is able to translocate into the nucleus actuating the transcription of several polypeptides, many of which have antioxidant capacity [46]. These includes catalase (CAT), which removes hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), which removes superoxide, and enzymes such as, heme oxygenase (HO-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), all of which can be affected by the cellular redox environ [47]. Further studies have also noted that hydrogen therapy has anti-apoptotic and anti-inflammatory effects in multiple disease models including respiratory, metabolic, and neurological diseases. [48-50]. Curiously, contemporary studies also note H_2 has various hormetic effects (51). For example, H_2 may initially enhance genetic expression of NFκB, an important transcription factor responsible for regulating the levels of proinflammatory molecules including the aforementioned cytokines, chemokines and hematopoietic growth factors [47]. Although the manner in which these processes occur has yet to be well defined, extended observations into the precise mechanism by which H_2 interacts with such proteins and peptides would be highly advantageous.

During times of cellular stress, particularly in viral and respiratory infection, leukocytes, principally macrophages and neutrophils, release toxic chemicals in an oxidative burst so as to inflict damage on the invading pathogen or dysfunctional cell. [52]. NOX complexes assemble on the membrane of the immune cell and force electrons (e^-) into the vacuole reducing O_2 to the superoxide anion ($O_2^{\cdot -}$) and altering pH via electron coupled translocation of protons within the phagolysosome. The oxidative burst, however, is not restricted to infected tissues and often results in not only the desired destruction of pathogens, but also injury to the adjacent healthy cells. This results in subsequent hyperinflammation and increased oxidation, underlying the pathological process that exacerbates severe COVID-19 infection. This contributes not only to enhanced ROS production, but also to increased bronchoalveolar lavage fluid (BALF), pulmonary infiltrates, and Type II cell hyperplasia as has been noted in clinical cases [39]. Type II cell hyperplasia is likely aggravated by the increase in activity of the mitogen-activated protein kinase (MAPK) pathway responsible for increasing genetic

transcription of growth factors. Significantly, H_2 has been noted to decrease protein levels of NFkB, p65, TNFa, IL-1b, and suppress various MAPK cascades involving ERK1/2, JNK, p38, etc. inhibit the activation of MAPKs cascades involving ERK, JNK, and p38 [53].

The inflammation process is an innate immune response that is possibly initiated and certainly agitated by any cellular redox imbalance. The innate response involves not only the lymphocytes previously mentioned in the contagion section above, but also the production and release of complement proteins, pro-inflammatory cytokines, and IgM antibodies, all of which contribute to the rapid production of ROS [53, 54]. An early study of Hepatitis B infection (HBV) by Xia *et al.* [55] demonstrated that administration of 1200-1800 mL per day of hydrogen-rich water (HRW), over six continuous weeks, reduced serum markers of oxidative stress (xanthine oxidase [XOD], malondialdehyde [MDA]) and increased antioxidant status (glutathione *S*-transferase [GST], SOD) [55]. Supporting evidence also details that inhalation of H_2 can specifically suppresses oxidative stress and inflammatory markers (MDA, GSH and IL-1, IL-6, TNF-α, CRP, respectively) associated with liver resection, in which ischemia reperfusion injury occurs [56]. These findings were confirmed both histologically and by direct measurement of oxidative stress in porcine models of disease [56].

Of importance to the global crisis created by the emergence of COVID-19 however, are the mounting reports that suggest application of H_2 in human respiratory diseases may modulate the acute and nocuous cytokine storm. This effectively diminishes the counterproductive inflammatory response and reduces parenchymal cell hyperplasia, which has been confirmed in multiple rodent models of ARDS, chronic obstructive pulmonary disorder (COPD), asthma and ventilator-induced lung injury [57-59]. Using similar rodent models, H_2 therapy has also been demonstrated to decrease airway resistance, reduce free radicals, and decrease dyspnoea [60]. Studies have further demonstrated that H_2 inhalation can ameliorate mucus production and reduce expression of cytokines IL-1β, IL-5, IFγ, and TNF-α [39, 47], and as such, H_2 could theoretically provide partial alleviation of the respiratory symptoms associated with hospitalization and mortality of COVID-19 patients.

As described in the previous section, excessive production of cytokines and chemokines are known to contribute to the inflammatory process, an action that in turn negatively influences enzymatic function in glycolytic pathways. Of relevance to the investigations involving the use of H_2 as an adjunctive therapy for severe respiratory disease is the mounting body of evidence suggesting H_2 has properties able to regulate many metabolic pathways [44, 61-63]. For example, Niu *et al*., reported that H_2 reverses the biological switch induced by inflammation from oxidative phosphorylation (oxphos), which produces substantial amounts of ATP within the mitochondria, to the less efficient but accelerated production of ATP via glycolysis [64], a phenomenon frequently described during infection and cellular stress events. Furthermore, metabolic dysregulation is known to provoke and intensify inflammatory responses that when working synergistically can cause a reduction in oxygen availability and subsequent hypoxia. The lack of biologically available O_2 further causes a shift from aerobic respiration to anaerobic respiration, which is an important factor for cellular energetics when fighting disease and viral infection [61,62]. Crucially, the conversion from oxphos to anaerobic glycolysis causes a reduction in the available ATP for the essential synthesis and activation of regulatory and defensive biological molecules, imperative in the defense against severe and acute respiratory infections.

Of interest for wider pulmonary conditions, H_2 has been noted to protect against cigarette smoke-induced pulmonary emphysema in mouse models [57]. Here H_2 ingestion was shown to reduce DNA damage, determined by a decrease in biological markers such as phosphorylated histone (H2AX) and 8-hydroxy-2-deoxyguanosine (8OHdG) and lower markers of senescence (e.g. β-galactosidase, p16 and p21), resulting in restored lung compliance [57]. Further studies into the consumption of HRW have delineated that molecular hydrogen increases the elimination of fine carbon particles from the lungs, thereby inhibiting lipid peroxidation of cellular membranes and attenuating lung injury [65]. Additionally, Terasaki *et al*., noted that consumption of HRW alongside inhalation of H_2 protects

against the long-term effects of pneumonitis in irradiated lung tissue via reduction of type III collagen deposition, negating the risk of inveterate tissue damage [66].

Proposed Delivery Mechanisms of H_2

Potentially, there are three routes for hydrogen administration in a clinical setting, ingestion of HRW, inhalation of H_2 gas, and intravenous infusion of hydrogen-rich saline (HRS). Of these possible application methods, infusion with HRS has been identified as having salubrious effects for numerous lung conditions including acute lung injury (ALI) and ARDS [67, 68], both of which are common in patients with severe COVID-19 infection [69]. Of particular interest are the investigations that demonstrate that H_2 is an anti-apoptotic, anti-inflammatory, anti-oxidative agent [2, 3]. Many of these reports describe significant reduction in proinflammatory cytokine production, reduced neutrophil infiltration and decreased DNA oxidation. This is accomplished through multiple mechanisms including the regulation of p38/MAPK and Bim/Bax activation that regulate inflammatory and apoptotic processes, respectively [68, 69]. Also of interest when analyzing the effects of H_2 on pulmonary disorders are the extensive reports that evidence induction of the cytoprotective P13K/Akt pathway that is responsible for the downstream production of important proteins such as claudin-5 protein, an adhesion molecule that protects against endothelial permeability, and aquaporins 1 and 5 that have an essential role in the evacuation of accumulative H_2O in ALI and ARDS [70-74].

Alternatively, hydrogen-rich water can be produced by infusing pure H_2 gas into water or beverages, or via electrolysis. It is also routinely formed by the reaction between non-ionic metallic magnesium (Mg) and H_2O, creating both magnesium hydroxide and molecular hydrogen according to the equation: $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$. Commercially there are various hydrogen products on the market ranging from portable tablets based on the magnesium reaction, which can deliver over 7 mg/L of H_2 [74], to ready-to-drink H_2 infused beverages [75]. There are also hydrogen water

machine technologies that can be large and static or incorporated into multiuse, small and portable bottles. However, customers should be aware that many products may either not perform as advertised and/or not provide doses of H_2 that are similar to what is used in clinical studies. Lastly, another method of administration is inhalation of H_2 gas, which allows conveyance directly into the pulmonary parenchyma and which can also be easily combined with O_2 therapy in an oxy-hydrogen mixture. Here, gaseous H_2 can be created either by utilizing technology that employs a proton exchange membrane requiring distilled water, or by way of electrolysis of H_2O enriched with electrolytes such as sodium/potassium hydroxide or bicarbonate. Alternatively, tanks of pre-mixed medical grade air containing 2-4% H_2, 21% O_2 and balance nitrogen are also used [76]. Regardless of the method, the gases can be delivered by way of a nasal cannula or facemask. This approach may allow clinicians to effectively administer both the oxygen needed to ensure optimal gas exchange within the lungs whilst also limiting the likelihood of hyperoxia-induced ROS/RNS accumulation via the aforementioned cytoprotective mechanisms. Hyperoxia is a condition that is known to intensify bronchopulmonary dysplasia in the epithelium, and of significance for COVID-19 patients, in type II alveolar cells [77]. Inhalation of H_2, either in conjunction with O_2, or through exclusive H_2 application, exhibits numerous cytoprotective qualities including upregulated expression of HO-1, Nrf2 and antioxidative compounds (e.g. SOD, CAT), activation of P13K/Akt downstream events, and inhibition of proinflammatory pathways involving NFκB, TNFα and IL-1β [77-79]. In a model of ventilator-induced lung injury Huang *et al.*, described a potential hormetic effect to H_2 gaseous employment, wherein H_2 application significantly reduced epithelial apoptosis by way of an initial enhancement of NFκB availability in the first hour post-administration; however, when tested two hours post-administration, NFκB levels were significantly reduced along with decreased levels of pro-apoptotic markers (e.g. BAX) and edema [79].

Conclusion and Recommendations

Although the primary targets of H_2 remain elusive, there is a rapidly expanding body of evidence detailing both the safety and efficacy of hydrogen treatment for numerous conditions. Of particular significance, due to the emergence of the novel SARS-CoV-2, are the reports that H_2 may have a salubrious effect in models of pulmonary inflammatory diseases, and those that detail abatement of the pernicious cytokine storm, conceivably through the reduction of deleterious ROS/RNS (e.g. $^{\cdot}OH$, $ONOO^-$) and regulation of multiple metabolic pathways. These are important factors in the pathogenesis of COVID-19, and amelioration of the redox environment may reduce the onset of severe symptomology in vulnerable patients.

Consequently, application of H_2, shown to be innoxious in analeptic doses, may provide an effective adjunctive medicament to O_2 inhalation in the treatment of COVID-19, for the critically ill. Although this method is recommended and practiced in the People's Republic of China [10] with oxygen/hydrogen mixed gas noted to significantly reduce dyspnoea [80], it is not widely used elsewhere. To date, only one clinical trial using oxyhydrogen for the treatment of COVID-19 infection has been registered with the US National Library of Medicine, with a further four clinical trials registered with The Centre for Evidence-Based Medicine (CEBM) [81]. Therefore, in the light of this unprecedented global pandemic, it would be prudent to further investigate the effect of molecular hydrogen, not only for the immediate threat of viral contagion, but also for other inflammatory respiratory conditions such as pneumonia, asthma, COPD and cystic fibrosis. As H_2 has also been demonstrated to work synergistically with $NO^{\bullet}$ [75] in combatting ROS overload and granulocyte infiltration during an animal model of cardiac infarction [82] it may be prudent to extend research into the combined effect of hydrogen therapy and other iatric gases.

It is the authors considered opinion that inhalation of H_2 would be more a more effective delivery mechanism for patients with moderate/severe symptoms of COVID-19. Also worthy of notation is that currently, most, but not all clinical trials have been based on inhalation of H_2, with this also being the preferred delivery method as recommended by The National Health Commission of the People's

Republic of China [10]. However, in some cases, such as Parkinson's Disease, HRW has been demonstrated as being more effective against disease progression [44]. Hence, the authors also suggest multiple clinical studies are required to determine the best method of H_2 administration.

Future research into the utility of H_2 within the clinical setting may also wish to reflect on the sustainability of hydrogen therapy with a view of employing this inexpensive and effective resource as an ancillary treatment for multiple pulmonary disorders.

CONFLICT OF INTEREST STATEMENT

The author confirms that they have no competing interests.

ACKNOWLEDGEMENTS

The authors would like to thank the University of the West of England, Bristol, for funding and literature access facilitating this research.

References

[1] Ishibashi, T. Therapeutic efficacy of molecular hydrogen: A new mechanistic insight. *Current Pharmaceutical Design*, **25**, 946-955 (2019).
[2] Ohta, S. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. *Pharmacology & Therapeutics*, **144**, 1-11 (2014).
[3] Ichihara, M., Sobue, S., Ito, M., Ito, M., Hirayama, M. and Ohno, K. Beneficial biological effects and the underlying mechanisms of molecular hydrogen-comprehensive review of 321 original articles. *Medical Gas Research*, **5**, 12 (2015).
[4] https://www.who.int/csr/sars/country/country2003_08_15.pdf?ua=1 [Accessed: 06/05/2020]
[5] de Groot, R.J., Baker, S.C., Baric, R.S., Brown, C.S., Drosten, C., Enjuanes, L., Fouchier, R.A., Galiano, M., Gorbalenya, A.E., Memish, Z.A., Perlman, S., Poon, L.L., Snijder, E.J., Stephens, G.M., Woo, P.C., Zaki, A.M., Zambon, M. and Ziebuhr, J. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *Journal of Virology*, **87**, 7790-7792 (2013).
[6] Liu, Y., Gayle, A.A., Wilder-Smith, A. and Rocklöv, J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *Journal of Travel Medicine*, **27**, taaa021 (2020).
[7] Rothan, H.A. and Byrareddy, S.N. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Journal of Autoimmunity*, **109**, 102433 (2020).

[8] Ohsawa, I., Ishikawa, M., Takahashi, K., Watanabe, M., Nishimaki, K., Yamagata, K., Katsura, K., Katayama, Y., Asoh, S. and Ohta, S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nature Medicine*, **13**, 688 (2007).
[9] Hu, T., Yang, M., Zhang, Z. Hydrogen Medicine Therapy: An Effective and Promising Novel Treatment for Multiple Organ Dysfunction Syndrome (MODS) Induced by Influenza and Other Viral Infections Diseases?*SOJ Microbiol Infect Dis* **5**, 1-6 (2017).
[10] Xu, Y., Chen, Y., and Tang, X. Guidelines for the diagnosis and treatment of coronavirus disease 2019 (COVID-19) in China. *Global Health & Medicine,* **2**, 66-72, (2020).
[11] Gorbalenya, A., Baker, S., Baric, R., de Groot, R., Drosten, C., Gulyaeva, A., Haagmans, B., Lauber, C., Leontovich, A., Neuman, B. and Penzar, D. Coronaviridae study group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology*, **2020**, 03-04 (2020).
[12] Cascella, M., Rajnik, M., Cuomo, A., Dulebohn, S.C. and Di Napoli, R. Features, Evaluation and Treatment Coronavirus (COVID-19). Statpearls. Treasure Island (FL): StatPearls Publishing LLC. (2020).
[13] Ren, L.L., Wang, Y.M., Wu, Z.Q., Xiang, Z.C., Guo, L., Xu, T., Jiang, Y.Z., Xiong, Y., Li, Y.J., Li, X.W., Li, H., Fan, G.H., Gu, X.Y., Xiao, Y., Gao, H., Xu, J.Y., Yang, F., Wang, X.M., Wu, C., Chen, L., Liu, Y.W., Liu, B., Yang, J., Wang, X.R., Dong, J., Li, L., Huang, C.L., Zhao, J.P., Hu, Y., Cheng, Z.S., Liu, L.L., Qian, Z.H., Qin, C., Jin, Q., Cao, B. and Wang, J.W. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chinese Medical Journal*, **133**, 1015-1024 (2020).
[14] Chan, J.F., Lau, S.K., To, K.K., Cheng, V.C., Woo, P.C. and Yuen, K.Y. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clinical Microbiology Reviews*, **28**, 465-522 (2015).
[15] Corman, V.M., Muth, D., Niemeyer, D. and Drosten, C. Hosts and sources of endemic human coronaviruses. Advances in Virus Research. Elsevier, pp.163-188 (2018).
[16] Mousavizadeh, L. and Ghasemi, S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. *Journal of Microbiology, Immunology and Infection* (in press) (2020).
[17] van Doremalen, N., Bushmaker, T., Morris, D.H., Holbrook, M.G., Gamble, A., Williamson, B.N., Tamin, A., Harcourt, J.L., Thornburg, N.J. and Gerber, S.I. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *New England Journal of Medicine*, **382**, 1564-1567 (2020).
[18] Wang, L., Wang, Y., Ye, D. and Liu, Q. A review of the 2019 Novel Coronavirus (COVID-19) based on current evidence. *International Journal of Antimicrobial Agents*, (in press) 105948 (2020).
[19] Lauer, S.A., Grantz, K.H., Bi, Q., Jones, F.K., Zheng, Q., Meredith, H.R., Azman, A.S., Reich, N.G. and Lessler, J. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Annals of Internal Medicine,* **172**, 577-582 (2020).
[20] Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X. and Shan, H. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology*. **158**, 1831-1833 (2020).
[21] Lai, C., Liu, Y.H., Wang, C., Wang, Y., Hsueh, S., Yen, M., Ko, W. and Hsueh, P. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARSCoV-2): Facts and myths. *Journal of Microbiology, Immunology and Infection* (in press) (2020).
[22] Shi, Y., Wang, Y., Shao, C., Huang, J., Gan, J., Huang, X., Bucci, E., Piacentini, M., Ippolito, G. and Melino, G., COVID-19 infection: the perspectives on immune responses. *Cell Death Differ*., **27,** 1451–1454 (2020).
[23] Goh, K.J., Choong, M.C., Cheong, E.H., Kalimuddin, S., Duu Wen, S., Phua, G.C., Chan, K.S. and Haja Mohideen, S. Rapid progression to acute respiratory distress syndrome: review of current

understanding of critical illness from COVID-19 Infection. *Ann. Acad. Med. Singapore*, **49**, 1-9 (2020).
[24] Ruan, Q., Yang, K., Wang, W., Jiang, L. and Song, J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Medicine*, **46**, 846-848 (2020).
[25] Luh, S. and Chiang, C. Acute lung injury/acute respiratory distress syndrome (ALI/ARDS): the mechanism, present strategies and future perspectives of therapies. *Journal of Zhejiang University Science B*, **8**, 60-69 (2007).
[26] Wang, Y., Kang, H., Liu, X. and Tong, Z. (2020) Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. *Journal of Medical Virology*, **92**, 538-539 (2020).
[27] https://www.worldometers.info/coronavirus/ [Accessed:06/05/2020]
[28] Sah, R., Rodriguez-Morales, A.J., Jha, R., Chu, D.K., Gu, H., Peiris, M., Bastola, A., Lal, B.K., Ojha, H.C., Rabaan, A.A. and Zambrano, L.I. Complete genome sequence of a 2019 novel coronavirus (SARS-CoV-2) strain isolated in Nepal. *Microbiology Resource Announcements*, **9**, e00169-20 (2020).
[29] Petrosillo, N., Viceconte, G., Ergonul, O., Ippolito, G. and Petersen, E. COVID-19, SARS and MERS: are they closely related? *Clinical Microbiology and Infection*. (in press) (2020).
[30] Zhang, H., Penninger, J.M., Li, Y., Zhong, N. and Slutsky, A.S. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Medicine*, **46**, 586–590 (2020).
[31] Kuba, K., Imai, Y., Ohto-Nakanishi, T., Penninger, J.M. Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacol. Ther*., **128**, 119-128 (2010).
[32] Meyerholz, D.K., Lambertz, A.M. and McCray Jr, P.B. Dipeptidyl peptidase 4 distribution in the human respiratory tract: implications for the Middle East respiratory syndrome. *The American Journal of Pathology*, **186**, 78-86 (2016).
[33] Wu, F., Zhao, S., Yu, B., Chen, Y., Wang, W., Song, Z., Hu, Y., Tao, Z., Tian, J. and Pei, Y. A new coronavirus associated with human respiratory disease in China. *Nature*. **579**, 265-269 (2020).
[34] Liu, Z., Xiao, X., Wei, X., Li, J., Yang, J., Tan, H., Zhu, J., Zhang, Q., Wu, J. and Liu, L. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. *Journal of Medical Virology*, **92**, 595-601 (2020).
[35] Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L. and Chen, H.D. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, **579**, 270-273 (2020).
[36] Shereen, M.A., Khan, S., Kazmi, A., Bashir, N. and Siddique, R. COVID-19 infection: origin, transmission, and characteristics of human coronaviruses. *Journal of Advanced Research*, **24**, 91-98 (2020).
[37] Leiva-Juárez, M.M., Kolls, J.K. and Evans, S.E. Lung epithelial cells: therapeutically inducible effectors of antimicrobial defense. *Mucosal Immunology*, **11**, 21-34 (2018).
[38] van der Vliet, A.. Nox enzymes in allergic airway inflammation. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1810**, 1035-1044 (2011).
[39] Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Sun, R., Tian, Z., Xu, X. and Wei, H. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *National Science Review*, **nwaa041** (2020).
[40] Abbas, A.K., Lichtman, A.H. and Pillai, S. Basic Immunology E-Book: Functions and Disorders of the Immune System. Elsevier Health Sciences (2019).
[41] Lambeth, J.D. and Neish, A.S. Nox enzymes and new thinking on reactive oxygen: a double-edged sword revisited. *Annual Review of Pathology: Mechanisms of Disease*, **9**, 119-145 (2014).

[42] Russell, G., Veal, D. and Hancock, J.T., Is glyceraldehyde-3-phosphate dehydrogenase a central redox mediator? *Reactive Oxygen Species*, **9**, 48-69 (2020).
[43] Vallee, Y., Shalayel, I., Ly, K.D., Rao, K.V.R., De Paepe, G., Marker, K. and Milet, A. At the very beginning of life on Earth: the thiol-rich peptide (TRP) world hypothesis. *The International Journal of Developmental Biology*, **61**, 471-478 (2017).
[44] Ohno, K., Ito, M., Ichihara, M. and Ito, M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. *Oxidative Medicine and Cellular Longevity*, **2012**, 353152, (2012).
[45] Dole, M., Wilson, F.R. and Fife, W.P. Hyperbaric hydrogen therapy: a possible treatment for cancer. *Science*, **190**, 152-154 (1975).
[46] Bhakkiyalakshmi, E., Sireesh, D. and Ramkumar, K.M. Redox Sensitive Transcription via Nrf2-Keap1 in Suppression of Inflammation. In*: Immunity and Inflammation in Health and Disease*. Elsevier,.149-161 (2018).
[47] Xiao, M., T. Zhu, T. Wang, and F. Q. Wen. Hydrogen-rich saline reduces airway remodeling via inactivation of NF-kappaB in a murine model of asthma. *Eur. Rev. Med. Pharmacol. Sci.*, **17**,1033-43 (2013).
[48] Yang, Y., Zhu, Y., and Xi, X. Anti-inflammatory and antitumor action of hydrogen via reactive oxygen species. *Oncology Letters*, **16**, 2771-2776. (2018).
[49] Liu, C.-l, Zhang, K., and Chen, G. Hydrogen therapy: from mechanism to cerebral diseases. *Medical Gas Research*, **6**, 48 (2016).
[50] Slezák, J., Kura, B., Frimmel, K., Zálešák, M., Ravingerová, T., Viczenczová, C., Okruhlicova, L. and Tribulová, N. Preventive and therapeutic application of molecular hydrogen in situations with excessive production of free radicals. *Physiological Research*, **65**, S11-S28 (2016).
[51] LeBaron, T.W., Laher I., Kura, B. and Slezak, J. Hydrogen gas: from clinical medicine to an emerging ergogenic molecule for sports athletes. *Canadian Journal of Physiology and Pharmacology*, **97**, 797-807 (2019).
[52] Warris, Adilia, and Elizabeth R. Ballou. "Oxidative responses and fungal infection biology." *Seminars in Cell & Developmental Biology*, vol. 89, pp. 34-46. Academic Press, (2019).
[53] Han, B., Zhou, H., Jia, G., Wang, Y., Song, Z., Wang, G., Pan, S., Bai, X., Lv, J. and Sun, B. MAPK s and Hsc70 are critical to the protective effect of molecular hydrogen during the early phase of acute pancreatitis. *The FEBS Journal*, **283**, 738-756 (2016).
[54] Halliwell, B. and Gutteridge, J.M. *Free Radicals in Biology and Medicine*. Oxford University Press, USA (2015).
[55] Xia, C., Liu, W., Zeng, D., Zhu, L., Sun, X. and Sun, X. Effect of hydrogen-rich water on oxidative stress, liver function, and viral load in patients with chronic Hepatitis B. *Clinical and Translational Science*, **6**, 372-375 (2013).
[56] Malý, O., Zajak, J., Hyšpler, R., Turek, Z., Astapenko, D., Jun, D., Váňová, N., Kohout, A., Radochová, V. and Kotek, J. Inhalation of molecular hydrogen prevents ischemia-reperfusion liver damage during major liver resection. *Annals of Translational Medicine*, **7**, 774 (2019).
[57] Lu, W., Li, D., Hu, J., Mei, H., Shu, J., Long, Z., Yuan, L., Li, D., Guan, R., Li, Y., Xu, J., Wang, T., Yao, H., Zhong, N. and Zheng, Z. Hydrogen gas inhalation protects against cigarette smoke-induced COPD development in mice. *Journal of Thoracic Disease*, **10**, 3232-3243 (2018).
[58] Zhang, B., Zhao, Z., Meng, X., Chen, H., Fu, G. and Xie, K. Hydrogen ameliorates oxidative stress via PI3K-Akt signaling pathway in UVB-induced HaCaT cells. *International Journal of Molecular Medicine*, **41**, 3653-3661 (2018).
[59] Qu, J., Li, Y., Zhong, W., Gao, P. and Hu, C. Recent developments in the role of reactive oxygen species in allergic asthma. *Journal of Thoracic Disease*, **9**, E32-E43 (2017).
[60] Bang, B.R., Chun, E., Shim, E.J., Lee, H.S., Lee, S.Y., Cho, S.H., Min, K.U., Kim, Y.Y. and Park, H.W. Alveolar macrophages modulate allergic inflammation in a murine model of asthma. *Experimental & Molecular Medicine*, **43**, 275-280 (2011).

[61] Ma, X., Ma, S., Xie, F., Zhang, Y., Yao, T., Liu, M., Zhang, X. and Zhao, P. Molecular hydrogen induces metabolic reprogramming to promote differentiation of glioma stem cells. (2020) DOI:10.21203/rs.3.rs-15407/v1. (Pre-peer review).
[62] Gray, C.T. and Gest, H. Biological formation of molecular hydrogen: A" hydrogen valve" facilitates regulation of anaerobic energy metabolism in many microorganisms. *Science*, **148**, 186-192 (1965).
[63] Kamimura, N., Ichimiya, H., Iuchi, K. and Ohta, S. Molecular hydrogen stimulates the gene expression of transcriptional coactivator PGC-1α to enhance fatty acid metabolism. *NPJ Aging and Mechanisms of Disease*, **2**, 1-8 (2016).
[64] Niu, Y., Nie, Q., Dong, L., Zhang, J., Liu, S.F., Song, W., Wang, X., Wu, G. and Song, D. Hydrogen attenuates allergic inflammation by reversing energy metabolic pathway switch. *Scientific Reports*, **10**, 1-13 (2020).
[65] Choi, J., Suk An, E., Ban Y.H., Woom Seo, D., Kim T.S., Lee S.P., Lin Y., Choi, E.K and Kim Y.B. Hydrogen-enriched water eliminates fine particles from the lungs and blood by enhancing phagocytic activity. *Journal of Biomedical Research*, **31**, 503 (2017).
[66] Terasaki, Y., Ohsawa, I., Terasaki, M., Takahashi, M., Kunugi, S., Dedong, K., Urushiyama, H., Amenomori, S., Kaneko-Togashi, M., Kuwahara, N., Ishikawa, A., Kamimura, N., Ohta, S. and Fukuda, Y. Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **301**, 415-426 (2011).
[67] Fang,Y., Fu, X.J., Gu, C., Xu, P., Wang, Y., Yu, W.R., Sun, Q., Sun, X.J. and Yao, M.. Hydrogen-rich saline protects against acute lung injury induced by extensive burn in rat model. *Journal of Burn Care & Research*, **32**, 82-91 (2011).
[68] Zhai, Y., Zhou, X., Dai, Q., Fan, Y. and Huang, X. Hydrogen-rich saline ameliorates lung injury associated with cecal ligation and puncture-induced sepsis in rats. *Experimental and Molecular Pathology*, **98**, 268-276 (2015).[69] Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S. and Chiumello, D. Covid-19 does not lead to a “typical” acute respiratory distress syndrome. *American Journal of Respiratory and Critical Care Medicine ja (in press)* (2020).
[70] Sun, Q., Cai, J., Liu, S., Liu, Y., Xu, W., Tao, H. and Sun, X. Hydrogen-rich saline provides protection against hyperoxic lung injury. *Journal of Surgical Research*, **165**, 43-49, (2011).
[71] Tao, B., Liu, L., Wang, N., Wang, W, Jiang, J. and Zhang, J. Effects of hydrogen-rich saline on aquaporin 1, 5 in septic rat lungs. *Journal of Surgical Research*, **202**, 291-298 (2016).
[72] Huang, C.S., Kawamura, T., Lee, S., Tochigi, N., Shigemura, N., Buchholz, B.M., Kloke, J.D., Billiar, T.R., Toyoda, Y. and Nakao, A. Hydrogen inhalation ameliorates ventilator-induced lung injury. *Critical Care*, **14**, 234 (2010).
[73] Wang, K., Song, X., Duan, S., Fang, W., Huan, X., Cao, Y., Tang, J. and Wang, L. Hydrogen-rich saline prevents the down regulation of claudin-5 protein in septic rat lung via the PI3K/Akt signaling pathway. *Int. J. Clin. Exp. Med*., **10**, 11717-11727 (2017).
[74] LeBaron, T.W., Larson, A.J., Ohta, S., Mikami, T., Barlow, J., Bulloch, J. and DeBeliso, M. Acute supplementation with molecular hydrogen benefits submaximal exercise indices. Randomized, double-blinded, placebo-controlled crossover pilot study. *Journal of Lifestyle Medicine*, **9**, 36-43, (2019).
[75] LeBaron, T.W., McCullough, M.L. and Ruppman Sr., K.H. A novel functional beverage for COVID-19 and other conditions: Hypothesis and preliminary data, increased blood flow, and wound healing. *J. Transl. Sci.* **6**. DOI: 10.15761/JTS.1000380.
[76] Tamura, T., Hayashida, K., Sano, M., Onuki, S., and Suzuki, M. Efficacy of inhaled HYdrogen on neurological outcome following BRain ischemia during post-cardiac arrest care (HYBRID II trial): study protocol for a randomized controlled trial. *Trials*, **18**, 488 (2017).

[77] Wu, D., Liang, M., Dang, H.,.Fang, F., Xu, F, and Liu, C. Hydrogen protects against hyperoxia-induced apoptosis in type II alveolar epithelial cells via activation of PI3K/Akt/Foxo3a signaling pathway. *Biochemical and Biophysical Research Communications*, **495**, 1620-1627 (2017).
[78] Suzuki, Y., Sato, T., Sugimoto, M., Baskoro, H., Karasutani, K., Mitsui, A., Nurwidya, F., Arano, N., Kodama, Y., Hirano, S.I., Ishigami, A., Seyama, K and Takahashi, K. Hydrogen-rich pure water prevents cigarette smoke-induced pulmonary emphysema in SMP30 knockout mice. *Biochemical and Biophysical Research Communications*, **492**, 74-81, (2017).
[79] Huang, C.S., Kawamura, T., Peng, X., Tochigi, N., Shigemura, N., Billiar, T.R., Nakao, A. and Toyoda, Y. Hydrogen inhalation reduced epithelial apoptosis in ventilator-induced lung injury via a mechanism involving nuclear factor-kappa B activation. *Biochemical and Biophysical Research Communications*, **408**, 253-258, (2011).
[80] Guan, W-j., Chen, R-c. and Zhong, N-s. Strategies for the prevention and management of coronavirus disease 2019. *European Respiratory Journal*, **55**, 2000597 (2020).
[81] https://www.clinicaltrials.gov/ct2/show/NCT04336462 [Accessed: 06/05/2020].
[82] Shinbo, T., Kokubo, K., Sato, Y., Hagiri, S., Hataishi R., Hirose, M., and Kobayashi, H. Breathing nitric oxide plus hydrogen gas reduces ischemia-reperfusion injury and nitrotyrosine production in murine heart. *American Journal of Physiology-Heart and Circulatory Physiology*, **305**, 542-550 (2013).
[83] https://www.cebm.net/covid-19-registered-trials-and-analysis/?fbclid=IwAR0s_4pr1Q9yMXneftD61Gp1B6OkSZEuG_EB0w9CtMklQhdKBQKkYw7xqY0 [Accessed:18/05/2020]
[84] https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2697049 [Accessed: 10/05/2020].
[85] Guo, Y.R., Cao, Q.D., Hong, Z.S., Tan, Y.Y., Chen, S.D., Jin, H.J., Tan, K.S., Wang, D.Y. and Yan, Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. *Military Medical Research*, **7**, 1-10 (2020).
[86] https://www.uniprot.org/ [Accessed:06/05/2020]

Tables

Table 1.

VIRUS CLASSIFICATION	
REALM	*Ribovria*
PHYLUM	*Incertae sedis*
ORDER	*Nidovirales*
FAMILY	*Coronaviridae*
GENUS	*Betacoronavirus*
SUBGENUS	*Sarbecovirus*
SPECIES	*SARS-related coronavirus*
STRAIN	*SARS-CoV-2*

Table 1: The ancestral taxa of SARS-CoV-2 novel coronavirus. Details retrieved from [84].

Figures and Legends:

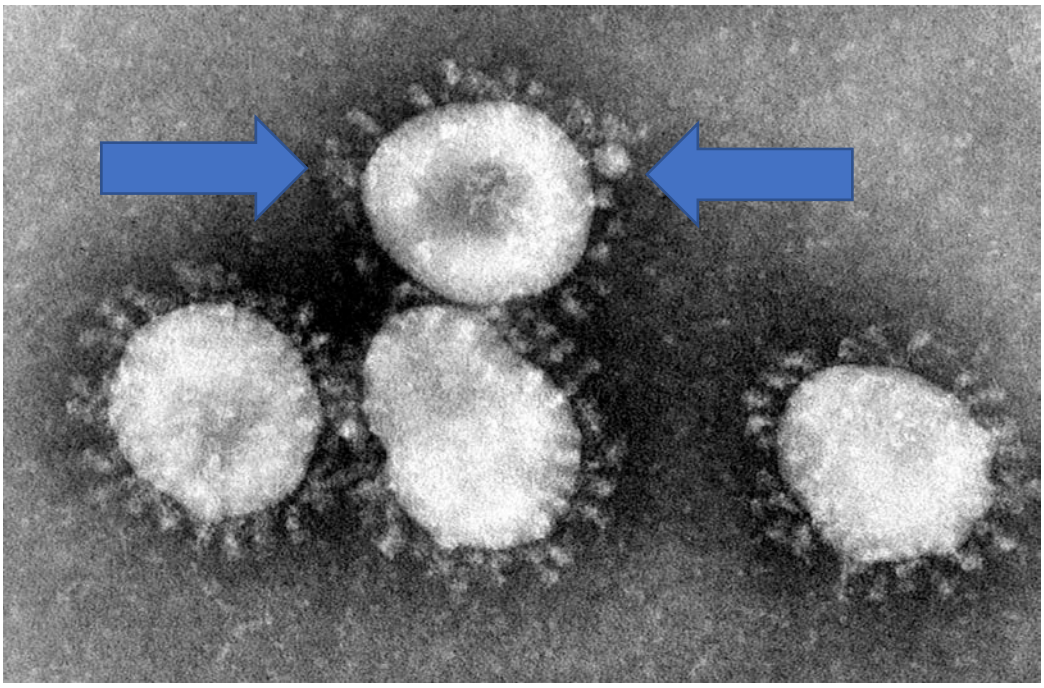

Figure 1: Electron micrograph of typical *corona viridae*. Blue arrow denotes the external glycoproteins that form a halo, or corona, around the capsid. Photo by Unknown Author is licensed under CC BY-SA Creative Commons.

Figure 2:

KEY:
SARS-CoV-2 (COVID-19)
SARS-CoV-1
Bat – HKU3

```
MFVFLVLLP----LVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFL 56
MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFL 60
MKILIFAFLANLAKAQEGCGIISRKPQPKMAQVSSSRRGVYYNDDIFRSDVLHLTQDYFL 60
* :::. :        . *  :        :  .*  ***** *.:***..*: *** **

PFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQS 116
PFYSNVTGFHTIN-------HTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQS 113
PFDSNLTQYFSLNVDS-DRYTYFDNPILDFGDGVYFAATEKSNVIRGWIFGSSFDNTTQS 119
** **:* :.:::         *.**:: * **:***:*****::***:**::::..:**

LLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL 176
VIIINNSTNVVIRACNFELCDNPFFAVSKPMG----TQTHTMIFDNAFNCTFEYISDAFS 169
AVIVNNSTHIIIRVCNFNLCKEPMYTVSR--G----TQQNAWVYQSAFNCTYDRVEKSFQ 173
:*:**:*:::*:.*:*::*.:*:  *    .     : .  ::..* ***:: :.. *

MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINIT 236
LDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINIT 229
LDTTPKTGNFKDLREYVFKNRDGFLSVYQTYTAVNLPRGLPTGFSVLKPILKLPFGINIT 233
:*   * ****.***:**** **:: :*. :  ::: *.** **..*:*:..**:*****

RFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPL 296
NFRAILTAFS------PAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPL 283
SYRVVMAMFS------QTTSNFLPESAAYYVGNLKYSTFMLRFNENGTITDAVDCSQNPL 287
::.::: .        : . :   :***:** *:  **:*:::**********: :**
```

```
775
776  SETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRK 356
777  AELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERK 343
778  AELKCTIKNFNVDKGIYQTSNFRVSPTQEVIRFPNITNRCPFDKVFNATRFPNVYAWERT 347
779  :* **::*.* ::*********** *: .::******* ***.:*****:* .****:*.
780
781  RISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTG 416
782  KISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTG 403
783  KISDCVADYTVLYNSTSFSTFKCYGVSPSKLIDLCFTSVYADTFLIRSSEVRQVAPGETG 407
784  :**:*****:*****: ********** :** ****:.****:*:::..:***:***:**
785
786  KIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAG 476
787  VIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPD 463
788  VIADYNYKLPDDFTGCVIAWNTAKHDT---G--NYYYRSHRKTKLKPFERDLSSDD---- 458
789  ********** ***:***: : *:   *  ** **  *: :*:*****:*.
790
791  STPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKN 536
792  GKPCTPP-ALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKN 522
793  ---------GNGVYTLSTYDFNPNVPVAYQATRVVVLSFELLNAPATVCGPKLSTELVKN 509
794           *  : *. *.*  .  :.**  **********:********* **:*:**
795
796  KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS 596
797  QCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVS 582
798  QCVNFNFNGLKGTGVLTSSSKRFQSFQQFGRDTSDFTDSVRDPQTLEILDISPCSFGGVS 569
799  :*********.****** *.*:*  ******* :* **:****:* *****:********
800
801  VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHV 656
802  VITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHV 642
803  VITPGTNASSEVAVLYQDVNCTDVPTAIRADQLTPAWRVYSTGVNVFQTQAGCLIGAEHV 629
804  *******:*.:***********:* .**:******:**:**** *****:**********
805
806  NNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPT 716
807  DTSYECDIPIGAGICASYHTVS----LLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPT 698
808  NASYECDIPIGAGICASYHTAS----VLRSTGQKSIVAYTMSLGAENSIAYANNSIAIPT 685
809  : ****************:* :      **...:**:********:.*:**:**:*****
810
811  NFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK 776
812  NFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDR 758
813  NFSISVTTEVMPVSMAKTAVDCTMYICGDSLECSNLLLQYGSFCTQLNRALTGIAIEQDK 745
814  **:**:***::****:**:***.******* **:*****************:*** ***:
815
816  NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQ 836
817  NTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQ 818
818  NTQEVFAQVKQMYKTPAIKDFGGFNFSQILPDPSKPTKRSFIEDLLFNKVTLADAGFMKQ 805
819  **:********:**** :* ************ **:*********************:**
820
821  YGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI 896
822  YGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQI 878
823  YGDCLGDVSARDLICAQKFNGLTVLPPLLTDEMVAAYTAALVSGTATAGWTFGAGAALQI 865
824  **:****: **********************:*:* **:**::** *:*************
825
826  PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNA 956
827  PFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNA 938
828  PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQESLSSTASALGKLQDVVNQNA 925
829  ************************ ******.**.:**:**::*::**************
830
831  QALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA 1016
832  QALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA 998
833  QALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA 985
834
835  ************************************************************
```

```
EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFT 1076
EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFT 1058
EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPSQEKNFT
1045************************************:*************:**:***

TAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNT 1136
TAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNT 1118
TAPAICHEGKAYFPREGVFVSNGTSWFITQRNFYSPQLITTDNTFVSGNCDVVIGIINNT 1105
*******:***:******** *** **:*****:.**:******************:***

VYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES 1196
VYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES 1178
VYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES 1165

************************************************************

LIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKF 1256
LIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGSCCKF 1238
LIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGSCCKF 1225

******************:***************:************.*********

DEDDSEPVLKGVKLHYT 1273
DEDDSEPVLKGVKLHYT 1255
DEDDSEPVLKGVKLHYT 1242
*****************
```

Figure 2. Amino acid sequence alignment of viral spike protein: In Black SARS-CoV-2 (Covid 19), in purple SARS-CoV-1 (SARS) and zoonotic, bat derived HKU3 in Green. Asterix (*) denotes no sequence change. (:) denotes a conserved change of amino acid in Covid 19 arrangement. (blank) denotes a change of amino acid across all three species (-) denotes omitted amino acids. Protein sequences obtained from uniport.org [86].

Figure 3a.

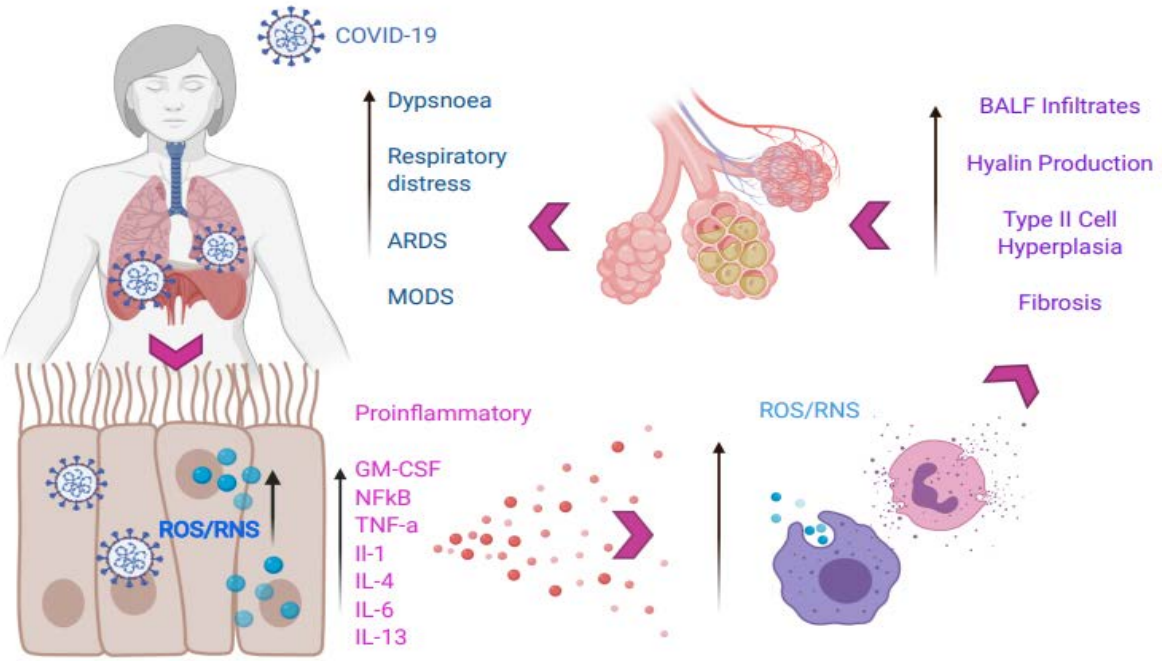

Figure 3a. Depicts cellular defense mechanisms triggered by SARS-CoV-2 infection that often result in the requirement for nosocomial interventions

Figure 3b

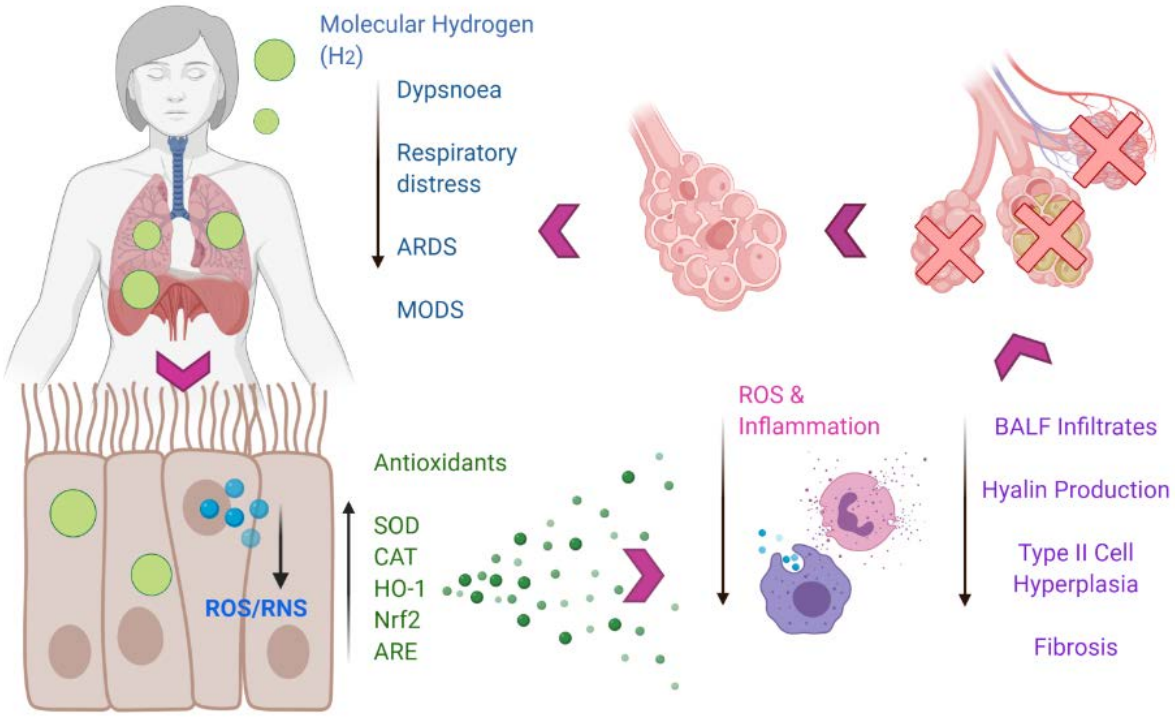

Figure 3b. Depicts the attenuation of proinflammatory factors that lead to a reduction of the severe symptoms associated with COVID-19.

SUPLLEMENTARY SECTION

Table 2.

Drug	Description	Action	Current usage	Citation
Lopinavir/Ritonavir	Protease inhibitors	Inhibits protease for protein cleavage, results in non-infectious, immature viral particles	HIV/AIDS, SARS, MERS	[i]
Chloroquine	9-aminoquinolin	Increases endosomal pH, immunomodulating, inhibits autophagy	Malaria, autoimmune disease	[ii]
Remdesivir (GS-5734)	Nucleotide analogue prodrug	Modulates virus post-entry	Ebola, SARS, MERS	[ii]
Nafamostat	Synthetic serine protease inhibitor	Prevents membrane fusion by reducing the release of cathepsin B; anticoagulant activities	Influenza, MERS, Ebola	[iii]
Ribavirin	Synthetic guanosine nucleoside	Interferes with the synthesis of viral mRNA	HCV, SARS, MERS	[iv]
Oseltamivir	Neuraminidase inhibitor	Prevents budding from the host cell, arrests viral replication, and infectivity	Influenza A	[v]
Penciclovir/ Acyclovir	Nucleoside analogue	A synthetic acyclic guanine derivative, results in chain termination	HSV, VZV	[vi]
Anakinra	Monoclonal antibody	IL-1α and IL-1β inhibition, results in reduction of hyperinflammatory response	In trial	[vii]
Tocilizumab	Monoclonal antibody	IL-6 inhibition and reduction of the hyperinflammatory response	Rheumatoid arthritis	[viii, ix]

Table 1. Current drug strategies for the treatment of COVID-19. Data adapted from [85].

Table References

[i] Chu, C.M., Cheng, V.C.C., Hung, I.F.N., Wong, M.M.L., Chan, K.H., Chan, K.S., Kao, R.Y.T., Poon, L.L.M., Wong, C.L.P., Guan, Y. and Peiris, J.S.M. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax,* **59**, 252-256 (2004).

[ii] Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W. and Xiao, G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Research*, **30**, 269-271 (2020).

[iii] Hsieh, H.P. and Hsu, J.T.A., 2007. Strategies of development of antiviral agents directed against influenza virus replication. *Current Pharmaceutical Design,* **13**, 3531-3542 (2007).

[iv] Arabi, Y.M., Shalhoub, S., Mandourah, Y., Al-Hameed, F., Al-Omari, A., Al Qasim, E., Jose, J., Alraddadi, B., Almotairi, A., Al Khatib, K. and Abdulmomen, A. Ribavirin and interferon therapy for critically ill patients with middle east respiratory syndrome: a multicentre observational study. *Clinical Infectious Diseases,* **70**, 1837-1844 (2020).

[v] McQuade, B. and Blair, M. Influenza treatment with oseltamivir outside of labelled recommendations. *American Journal of Health-System Pharmacy,* **72**, 112-116 (2015).

[vi] Shiraki, K. Antiviral drugs against alpha herpesvirus. In *Human Herpesviruses*, 103-122. *Springer, Singapore* (2018).

[vii] King, A., Vail, A., O'Leary, C., Hannan, C., Brough, D., Patel, H., Galea, J., Ogungbenro, K., Wright, M., Pathmanaban, O. and Hulme, S. Anakinra in COVID-19: important considerations for clinical trials. *The Lancet Rheumatology,* in press (2020).

[viii] Fu, B., Xu, X. and Wei, H. Why tocilizumab could be an effective treatment for severe COVID-19? *Journal of Translational Medicine*, **18**, 1-5 (2020).

[ix] Luo, P., Liu, Y., Qiu, L., Liu, X., Liu, D. and Li, J. Tocilizumab treatment in COVID-19: A single centre experience. *Journal of Medical Virology*, in press (2020).