An Overview of SARS-CoV-2 (COVID-19) Infection: The Importance of Molecular Hydrogen as an Adjunctive Therapy

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ABSTRACT | SARS-CoV-2 is an emerging β-coronavirus that causes COVID-19 disease, manifesting 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 (H2) 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 H2 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.

KEYWORDS | COVID-19; Cytokine storm; Molecular hydrogen; Reactive oxygen species; SARS-CoV-2; Virus

ABBREVIATIONS | ACE2, angiotensin converting enzyme-2; ARDS, acute respiratory distress syndrome; ATP, adenosine triphosphate; BALF, bronchial alveolar lavage fluid; CAT, catalase; CDC, Centers for Disease Control and Prevention; 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, reduced glutathione; GST, glutathione S-transferase; H2, molecular hydrogen; HBV, hepatitis B virus; HO-1, heme oxygenase-1; 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,
1. INTRODUCTION

Molecular hydrogen (H\textsubscript{2}) is an uncharged, non-polar, diatomic molecule with a low molecular weight of 2.016 g/mol. These characteristics make H\textsubscript{2} highly favorable for medicinal use as they allow H\textsubscript{2} to diffuse through both the membrane of the cell and those that occur around organelles, such as mitochondria \cite{1, 2}. The distribution of H\textsubscript{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\textsubscript{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 \cite{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 \cite{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% \cite{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 a 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 31\textsuperscript{st} December 2019, and within the month the World Health Organization (WHO) declared a public emergency \cite{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°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 H2 therapy attenuates the damage to alveolar 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 H2 utilization in a clinical setting is the selective ROS antioxidant property, 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 (OH\textsuperscript{•}) and peroxynitrite (ONOO\textsuperscript{−}) molecules, but no reduction in the important signaling molecules hydrogen peroxide (H2O2) and nitric oxide (NO\textsuperscript{•}) [8]. Correspondingly, it is proposed that due the ability to diffuse through biological membranes coupled with both anti-inflammatory and selective antioxidant properties, H2 may dampen the effects of the damaging cytokine storm [9], underlying 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 has provided a detailed intervention strategy that includes administration of various antiviral medications (e.g., lopinavir, ritonavir, chloroquine phosphate) along with inhalation of molecular hydrogen (66:33%; H2:O2) as an adjunctive therapeutic that can be delivered through nasal cannulas [10]. 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 H2 may affect cellular responses during severe SARS-CoV-2 infection.

2. 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 city 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, according to the International Virus Classification Commission, in

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.
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, and 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].

### 3. TRANSMISSION, INCUBATION, AND SYMPTOMATOLOGY

In line with many respiratory virus transmission patterns, SARS-CoV-2 is passed directly through aero-

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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, dyspnea, 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 which 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, and bronchial alveolar lavage fluid (BALF) [26], all of which are parameters that undoubtedly increase the mortality rate for this particular infection.

4. 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 (6.13%), when compared with Africa (3.09%) or Asia (2.97%).

5. 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, while 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 utilize as a receptor the angiotensin converting enzyme-2 (ACE2), a carboxypeptidase located in the airway epithelium and in lung parenchymal tissues [30, 31]. The (S) protein of SARS-

FIGURE 2. Amino acid sequence alignment of viral spike protein. SARS-CoV-2 (Covid-19) in black, SARS-CoV-1 (SARS) and zoonotic in purple, and 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; and (−) denotes omitted amino acids. Protein sequences obtained from https://www.uniprot.org
Cov-2 is comprised of two subunits. The outer S1 subunit initiates adhesion to the host cell while 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 while 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 birds, hedgehogs, snakes, turtles, or protected pangolins, all of which were noted to have been commercially available in the city of Wuhan [33]. Supporting evidence for the likelihood of bats as the natural hosts of SARS-CoV-2 was published in Nature by Zhou et al. who demonstrated a 96.2% sequence similarity with BatCoV RaTG13 (GISAID accession: EPI_ISL_402131), a virus whose natural hosts are vespertilio, or bat, species [35]. Ongoing research by Shereen and colleagues suggests that SARS-CoV-2 has higher transmission rate when compared with SARS-CoV-1. This has been considered to be a result of genetic recombination of the (S) protein in the RBD region of the SARS-CoV-2 virus, which is further hypothesized to explain the virus’ increased ability to adhere to the human ACE2 receptor [36].

6. THE IMMUNE RESPONSE TO COVID-19 AND ROS INVOLVEMENT

During SARS-CoV-2 infection, as with other respiratory infections, the primary epithelial response is to produce and express physiological distress proteins such as cytokines, chemokines, and growth factors. These include those of the interleukin family whose role is to direct and recruit leukocytes to the site of infection [37]. Inflammatory mediators also increase the production of ROS within the mitochondria. Notably, ROS are also produced by way of upregulated expression of NOX enzymes (e.g., NOX2) responsible for generation of these oxidative entities during the respiratory bursts from recruited phagocytes [38]. This can proceed in a cyclical manner, wherein cytokines upregulate ROS activity and elevated ROS lead to an increase in cytokine expression. This is the proposed mechanism by which the extensive and life-threatening parenchymal tissue damage occurs in COVID-19 patients [22], illustrated in Figure 3.

Once the cell membrane has been breached, the host cell has been demonstrated to respond by activating CD4+ lymphocytes that rapidly differentiate into pathogenic, hyper-inflammatory T-helper cells that express proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and various 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 oxidases (e.g., 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, cNOS) and dual oxidase (DUOX1/2) also contribute to increasing ROS/RNS during cellular stress events [41]. Intense and acute elevation of ROS/RNS causes damage to cellular structures via initiation of free-radical cascades, breaching phospholipid membrane integrity; such an elevation of reactive electrophilic species can also be responsible for the modification of essential metabol-
ic 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.

7. 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 O₂ 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 H₂ should be used in combination with other treatments such as anakinra (an IL-1 receptor antagonist) or tocilizumab (an IL-6 inhibitor), or in conjunction with O₂, considering H₂ is likely to be a complimentary, restorative molecule. Equally, since H₂ 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.

8. H₂—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 (H₂) with a molecular weight of 2.016 g/mol [43]. As a result of its low molecular weight, small size, and nonpolar nature, H₂ is highly diffusible and able to permeate through the blood/brain barrier, lipid membranes, cytosolic fluid, and into the cellular organelles. These properties of H₂ are deemed profoundly favorable considering conventional antioxidants lack these abilities, and are therefore, likely to be therapeutically less effective [44].

The salutary qualities of H₂ were first recognized by Dole and colleagues (1975) who demonstrated that hyperbaric hydrogen therapy, with a dosage of 2.5% O₂:97.5% H₂ 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 H₂ had a selective antioxidant effect, favoring reduction of non-signaling ROS/RNS, that the molecular mechanisms of H₂ activity began to be elucidated.

Ongoing research into this area has revealed that H₂ 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 genes, and many of the gene products have antioxidant capacity [46]. These includes catalase (CAT), which removes hydrogen peroxide (H₂O₂); superoxide dismutase (SOD), which removes superoxide; and multiple other 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 that H₂ has various hormetic effects [51]. For example, H₂ may initially enhance genetic expression of NF-κB, an important transcription factor responsible for regulating the expression 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 H₂ 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\textsubscript{2} to the superoxide anion (O\textsubscript{2}⁻) 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 that underly the pathological process, exacerbating severe COVID-19 infection. This contributes not only to enhanced ROS production, but also to increased BALF, pulmonary infiltrates, and type II cell hyperplasia as 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\textsubscript{2} has been noted to decrease protein levels of, p65, TNF-\textgreek{a}, and IL-1\textbeta, and suppress various MAPK 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\textsubscript{2} can specifically suppress oxidative stress and inflammatory markers (MDA, GSH and IL-1, IL-6, TNF-\textgreek{a}, 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 that application of H\textsubscript{2} in human respiratory diseases may modulate the acute and noxious 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\textsubscript{2} therapy has also been demonstrated to decrease airway resistance, reduce free radicals, and decrease symptomatic dyspnea [60]. Studies have further demonstrated that H\textsubscript{2} inhalation can ameliorate mucus production and reduce expression of cytokines IL-1\textbeta, IL-5, IF\gamma, and TNF-\textgreek{a} [39, 47], and as such, H\textsubscript{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 is 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\textsubscript{2} as an adjunctive therapy for severe respiratory disease is the mounting body of evidence suggesting that H\textsubscript{2} has properties able to regulate many metabolic pathways [44, 61–63]. For example, Niu et al. reported that H\textsubscript{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\textsubscript{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\textsubscript{2} has been noted to protect against cigarette smoke-induced pulmonary emphysema in mouse models [57]. Here H\textsubscript{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 (8OH-dG) 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 H₂ 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 H₂ 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].

9. PROPOSED DELIVERY MECHANISMS OF H₂

Potentially, there are three routes for hydrogen administration in a clinical setting, i.e., ingestion of HRW, inhalation of H₂ 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 H₂ 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 H₂ on pulmonary disorders are the extensive reports that evidence induction of the cytoprotective PI3K/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 H₂O in ALI and ARDS [70–74].

Alternatively, HRW can be produced by infusing pure H₂ gas into water or beverages, or via electrolysis. It is also routinely formed by the reaction between non-ionic metallic magnesium (Mg) and H₂O, creating both magnesium hydroxide and molecular hydrogen according to the equation: Mg + 2H₂O → Mg(OH)₂ + H₂. Commericially 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 H₂ [74], to ready-to-drink H₂ 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 or not provide doses of H₂ that are similar to those used in clinical studies.

Lastly, another method of administration is inhalation of H₂ gas, which allows conveyance directly into the pulmonary parenchyma and which can also be easily combined with O₂ therapy in an oxy-hydrogen mixture. Here, gaseous H₂ can be created either by utilizing technology that employs a proton exchange membrane requiring distilled water, or by way of electrolysis of H₂O enriched with electrolytes such as sodium/potassium hydroxide or bicarbonate. Alternatively, tanks of pre-mixed medical grade air containing 2–4% H₂, 21% O₂, 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 while 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 H₂, either in conjunction with O₂, or through exclusive H₂ application, exhibits numerous cytoprotective qualities including upregulated expression of HO-1, Nrf2, and antioxidative molecules (e.g., SOD, CAT), activation of PI3K/Akt downstream events, and inhibition of proinflammatory pathways involving NF-kB, TNF-α, and IL-1β [77–79]. In a model of ventilator-induced lung injury Huang et al. described a potential hormetic effect to H₂ gaseous employment, wherein H₂ application significantly reduced epithelial apoptosis by way of an initial enhancement of NF-kB availability in the first hour post-administration; however, when tested two hours post-administration, NF-kB levels were significantly reduced along with decreased levels of pro-apoptotic markers (e.g., BAX) and edema [79].
10. CONCLUSION AND RECOMMENDATIONS

Although the primary targets of H2 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 H2 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 (such as OH- and 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 H2, shown to be innoxious in analeptic doses, may provide an effective adjunctive medicament to O2 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 dyspnea [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 H2, 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 H2 has also been demonstrated to work synergistically with NO• [75] in combating 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’ opinion that inhalation of H2 would be 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 H2, 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 of this review article also suggest that multiple clinical studies are required to determine the best method of H2 administration. Future research into the utility of H2 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.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES


