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RESEARCH ARTICLE

The Influence of Molecular Hydrogen Therapies in Managing the Symptoms of Acute and Chronic COVID-19

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ABSTRACT

Coronavirus Infectious Disease 2019 (COVID-19) is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) that emerged as a novel pathogen of global concern in the latter stages of 2019. COVID-19 is a highly contagious disease which can be transmitted through aerosol droplets and surface-to-host contact. Both symptomology and the severity of disease can vary wildly between individuals, from asymptomatic but infectious, to those that require critical care. Due to the neoteric emergence of SARS-CoV-2, current treatment strategies are not yet well developed and rely on the repurposing of such medications as antiviral, corticosteroid, immunosuppressant and oxygen (O_2) therapies. However, the minimal efficacy of these interventions is concerning. In addition to the acute infection that prevails, it is estimated that up to 30% of adults who contract COVID-19 develop chronic symptoms lasting longer than 12 weeks. It is also estimated that 15% of children aged 2-16 years have developed long-lasting sequelae associated with SARS-CoV-2 infection.

According to recent clinical data, molecular hydrogen (H_2) and oxy-hydrogen (H_2/O_2) therapies successfully remediated the debilitating effects of SARS-CoV-2 infection in adults. By acting as an effective anti-inflammatory and antioxidative agent, it is reported that H_2 administration can improve recovery through abatement of the hyperinflammatory cytokine cascade and reduction of inhalation resistance in patients with mild-moderate disease symptoms.

In this review, the authors investigate the clinical and empirical evidence relating to treating the symptoms of both acute and chronic COVID-19 with H_2-containing therapeutics.

Abbreviations:

Alanine Aminotransferase (ALT); Alkaline Phosphatase (ALP); Catalase (CAT); Chronic Obstructive Pulmonary Disease (COPD); Coronavirus Infectious Disease 2019 (COVID-19); C-reactive protein (CRP); Creatinine Kinase (CK); Gamma-Glutamyl Transferase (GGT); Glutathione Peroxidase (GPx); Hydrogen-Rich Saline (HRS); Hydrogen-Rich Water (HRW); Lactate Dehydrogenase (LDH); Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV); Multisystem Inflammatory Syndrome in Children (MIS-C); Myalgic Encephalomyelitis Chronic Fatigue Syndrome (ME-CFS); Nuclear Erythroid Factor-2 (Nrf-2); Oxidative Stress (OxS); Receptor Binding Domain (RBD); Reactive Oxygen and Nitrogen Species (ROS/RNS); Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV); Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2); Superoxide Dismutase (SOD); World Health Organisation (WHO)

Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) is a positive sense, single-stranded, RNA (+ ssRNA) virus and the causative agent of Coronavirus Infectious Disease 2019 (COVID-19). After emerging in the concluding months of 2019, SARS-CoV-2 was shown to be highly contagious, spreading via aerosol transmission and rapidly disseminated around the globe.[1,2] The World Health Organisation (WHO) declared COVID-19 an international pandemic a few months later, 11th of March 2020.[3] Although approximately 80% of people who contracted COVID-19 in the first instance were asymptomatic, or experienced mild flu-like symptoms,[4] the initial wave of disease caused a significant burden for healthcare industries as 15% of patients required nosocomial care, and a further 5% needing critical ministrations.[5] Considering patient well-being and the impact this has on global socioeconomic factors, a report commissioned by the WHO in February of 2020 declared the median time for clinical recovery in mild cases of COVID-19 was 14 days, whilst analysis of recovery rates in severe or critical illness found 21-42 days were more typical.[6] In symptomatic patients, COVID-19 typically presents with episodes of continuous, non-productive coughing, fever, a loss of olfactory and gustatory senses, and musculoskeletal and respiratory distress.[7] It is, however, the complications that can arise from acute respiratory distress and hyperinflammatory responses that are most concerning for healthcare professionals as these can lead to critical illness, particularly in the elderly, immunocompromised and otherwise medically vulnerable demographic. Even with the advent of vaccines such severe reactions to infection are problematic, in part due to the issues surrounding accessibility to and availability of immunization,[8] and in part due to the waning efficacy of vaccines[9] in light of emergent SARS-CoV-2 variants.

In March 2020 anecdotal evidence began suggesting that an abstruse and protracted convalescent period was prevalent in some individuals recovering from COVID-19, with postliminary evidence suggesting that dyspnoea, fatigue, lapses in concentration and a persistent cough (29%, 58%, 27%, 43%, respectively) are commonly associated with an extended recuperation.[10] The pervasiveness of this condition, although problematic to diagnose due to lack of positive serological evidence of infection, is higher in nosocomial patients (87%) than in outpatients (35%),[10] suggesting that clinical renascence is likely to be associated with the asperity of primary disease. Moreover, in the general population, the lasting residuum of COVID-19 is estimated to be 4.4% in children,[11] 10% in adults aged 18-49, 15% in females aged 50-60 years, 10% in males aged 50-60 years and 22% in individuals over the age of 70 years.[12] In the light of developing knowledge regarding the effects of both short-term and long-term COVID-19, the National Institute for Health and Care Excellence (NICE), U.K.,[13] have now reassessed classification terms for illness associated with SARS-CoV-2 infections coining the terms 'Acute COVID-19' for the severe pathosis associated with primary infection, 'Ongoing Symptomatic COVID-19' for symptoms persisting for between 28 and 84 days, and 'Post-COVID-19 Syndrome' for infirmity lasting longer than 12-weeks (84-days).[13] When comparing the symptoms of acute COVID-19 with those of post-COVID-19 syndrome there is a clear delineation between the manifestation of symptoms during primary infection and the residual contrecoup experienced during post-COVID-19 rehabilitation. For example, anosmia, enteric disturbance and fever are largely resolved in post-COVID-19 syndrome. However, nascent symptoms including cardiac syndrome, fatigue and abatement of neurological function are frequently recounted as long-lasting symptoms.[4,8,14]

Research into molecular hydrogen (H_2), the paired aggregate of atomic hydrogen (H), demonstrates numerous salutary effects including anti-apoptotic, analgesic, anti-inflammatory and antioxidative qualities,[15] which are likely to be advantageous in the treatment of COVID-19 and other inflammatory-related conditions. H_2 is a gaseous substance and, therefore, easily incorporated into biological functions. H_2 is a diatomic molecule with a low molecular weight of 2.016 g/mol, which is non-polar and electrochemically neutral.[1] Due to these characteristics, the distribution of H_2 across phospholipid membranes is not affected by electrochemical gradients or hydrophilic and, or hydrophobic forces. Therefore, H_2 can greatly influence reactions in the cytosol and within discrete cellular compartments that include organelles such as the mitochondria. Research into H_2 as an interventional therapy for a wide range of infectious and non-infectious diseases is developing rapidly, with a growing number of laboratory[15-18] and clinical investigations[19-22] attesting to the safety and efficacy of H_2-inclusive treatments. H_2 as a medicament is versatile as the gas can be administered in various ways including through the inhalation of gas, in either isolation or in

combination with other gases; infusion of hydrogen-rich saline (HRS); ingestion of hydrogen-rich water (HRW); or topical application.[23]

As knowledge of the after-effects of SARS-CoV-2 infection is still developing, this review examines the recent scientific literature surrounding the evolution of SARS-CoV-2 and the divergent symptoms of post-COVID-19 syndrome, offering a potentially expedient solution to both the astringent consequences of acute COVID-19 and the long-lasting complications that can occur as a result of. With such a simplistic and diverse range of application methods available, interest in H_2 therapeutics is rapidly gaining momentum and 15-years of objective analysis strongly demonstrates that H_2 is a notably effective anti-inflammatory and antioxidant compound.[15-26] Hence, the authors have conducted a hermeneutic exposition into whether emerging viral lineages are likely to impact the duration and severity of COVID-19 illness and how H_2 therapeutics can be utilised in the symptomatic treatment of both acute COVID-19 and post-COVID-19 syndrome.

SARS-CoV-2 Viral Variants

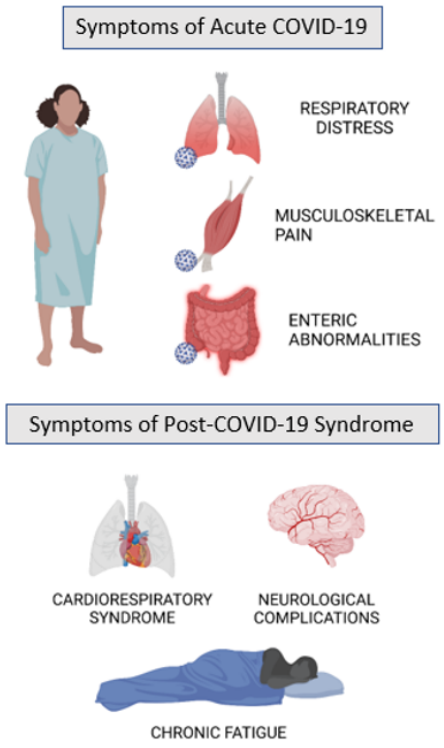
It is now well regarded that the identifying symptoms of SARS-CoV-2 infection changed,[27-31] and that these changes are likely to correspond with mutagenic adaptations in the viral genome but what has not been well investigated is whether such adaptations also have an effect on the long-lasting ramifications known as post-COVID-19 syndrome. To elucidate, the three main symptoms of both wild-type and alpha variant infections which circulated early in the pandemic present as fever, a persistent and continuous cough, and loss of taste (ageusia) and, or smell (anosmia).[28] However, the delta variant, which became the prevalent viral strain almost a year later, was determined to be 50% more infectious and more likely to present with upper respiratory and flu-like symptoms, replacing the persistent cough and ageusia/anosmia as the most common symptoms of acute COVID-19.[28] Consequently, it is of vital importance for collective welfare that prevention strategies at international, national and regional levels consider the extent to which SARS-like viruses evolve if long-lasting prophylaxis and effective treatment methods are to be developed.

Current Treatments and Vaccine Prophylaxis

As of May 2022, it is estimated that 1 billion people in low-to-middle income countries remain unvaccinated, whilst only 57 countries of 193 have reported vaccine fidelity in >70% of the population.[8] These figures are concerning as vaccination can radically reduce both the transmission of SARS-CoV-2 and individual susceptibility to the severe corollary of COVID-19.[7-9,32,33] However, single vaccine doses are not sufficient to induce lasting immunological aegis and multiple doses of each available vaccine are required. Therefore, there is still a pressing requirement for accessible, affordable and effective therapeutics which reduce the burden of COVID-19-related disease.

Current treatment strategies for moderate and severe COVID-19 illness have relied upon repurposing of antiviral, corticosteroid and monoclonal antibody drugs, Figure 1., alongside interventional O_2 therapies.[34-42] Of these treatments, the corticosteroid Dexamethasone has shown to be effective in reducing the serious corollary associated with hypersensitive immune reactions and the pernicious cytokine storm,[38] however extended use is not recommended due to consequent inhibition of the adaptive immune response.[38,39] Similarly, utilising antiviral agents has been shown to have undetermined efficacy.[34-37] Furthermore, there are no evidence-based treatment options for post-COVID-19 syndrome and further research into effective therapeutics for this often debilitating condition is imperative.

Figure 1. Symptoms and Treatment of COVID-19-associated Conditions



Acute COVID-19		
Grouping:	Symptoms:	Current Treatment:
Respiratory	Cough, Dyspnoea, Fever	O_2, Immune inhibitors, Corticosteroids
Musculoskeletal	Myalgia	Analgesics
Enteric	Abdominal pain, Emesis, Gastrointestinal Dysfunction	Analgesics, Anti-emetics, Nutritional Supplements
Post-COVID-19 Syndrome		
Grouping:	Symptoms:	Current Treatment:
Cardiorespiratory Syndrome	Cardiac Palpitations, Cough, Dyspnoea	Nutritional Supplements, Physical Therapy
Chronic Fatigue	Fatigue, Myalgia, Sleep Disturbances	Nutritional Supplements, Physical Therapy, Sleeping Tablets
Neurological	Anxiety, Depression, Impaired Cognition	Cognitive Behavioural Therapy

Legend: Graphical and scriptural comparison of acute and chronic COVID-19 symptoms alongside current treatment options. Graphics created by authors in BioRender.com.

Molecular Hydrogen (H_2) – Safety Aspects

Biological Tolerance

Molecular hydrogen (H_2) is a typically inert, colourless, non-toxic, odourless and tasteless gas that acts as a natural and novel anti-inflammatory and antioxidant. H_2 is naturally produced through fermentation of nutrients by intestinal micro-organisms such as *Clostridia* and *Coliform* species of bacteria.[43] Endogenous H_2, however, is not found in significant quantities to be therapeutically advantageous and recent research has shown inhalation of H_2 gas to be beneficial for a range of human ailments. To exemplify, high exposure to H_2 (96%), in conjunction with 4% oxygen (O_2), has been used as a treatment to prevent decompression sickness[44] in deep-sea divers since 1944 with no adverse effects. Reporting of side-effects, known as adverse events, is a mandatory requirement for clinical trial registration and market acceptance of new devices and products. Recent Clinical Trials have investigated the safety and effectiveness of oxy-hydrogen inhalation in combatting the severe symptoms associated with such diseases as Cancer[45] (NCT03818347), COPD[46] (NCT04000451) and COVID-19 (NCT04378712).[47] No serious or long-lasting side-effects are reported in any of the data regarding hydrogen-related therapies.

Safety

In its gaseous state H_2 has a flammability range of 4% – 94% at standard pressure and temperature.[48] Therefore calculations assessing the inhalation volume of H_2 should be conducted before administration. For example, if a patient is experiencing rapid or shallow breathing, or has a chronic lung condition, it would be beneficial to conduct a simple spirometry test to ensure accurate measurement of tidal volume and H_2 consumption, using the formula:

(mL/sec): H_2 / (Breath – H_2) x 100

H_2 is classified as a 'generally regarded as safe' (GRAS) product by the Food and Drug Administration (U.S.),[49] and is regarded as a food supplement (E949) under part C group I of regulation 1129/2011 in the European Union [50,51] and U.K.[52]

Molecular Hydrogen (H_2) as an Antioxidant in the Treatment of Acute COVID-19

One of the primary clinical functions of H_2 is as an antioxidant, first demonstrated by Ohsawa *et al.* (2007) in a rodent model of ischemia/reperfusion injury,[15] an event known to cause an overwhelming increase in localised generation of ROS/RNS. The seminal investigations describe selective reduction of the highly reactive hydroxyl radical ($^{\bullet}OH$) and peroxynitrite ($ONOO^-$) molecules, but no reduction in the important redox signalling molecules hydrogen peroxide (H_2O_2) and nitric oxide ($NO^{\bullet}$). This mechanism effectively allows the cell to upregulate expression of antioxidant enzymes (e.g., CAT, SOD) and transcription factors (e.g., nuclear erythroid factor-2, Nrf-2) that work synergistically to restore cellular homeostasis. In addition to the antioxidant and anti-inflammatory benefits proffered through regulation of gene expression, H_2 also has modulatory effects on the production of $NO^{\bullet}$ which is an essential immunomodulatory molecule responsible for the somatic defence against viral pathogens, wherein it acts as an effective antithrombotic factor and vasodilator.[53] However, excessive $NO^{\bullet}$ production when accompanied by an increase in $O_2^{\bullet-}$ production, leads to formation of the cytotoxic $ONOO^-$ anion, diminishing the levels of $NO^{\bullet}$ available for biologically advantageous processes. Such fundamental biochemistry has a major influence on the pathogenesis of COVID-19 and the progression into critical illness causing irreversible damage to blood-vessel epithelia and pneumocytes, effectively contributing to the inflammatory process and impairing O_2 exchange. In patients experiencing moderate-severe symptoms of COVID-19 reduced levels of O_2 saturation (SpO_2) can lead to hypoxaemia and tissue hypoxia,[54] driving the progression of illness towards Acute Respiratory Distress Syndrome (ARDS) and, or Multiple Organ Dysfunction Syndrome (MODS). Current medical interventions rely on increasing O_2 levels through either high-flow O_2 therapy or invasive mechanical ventilation,[55] or by repurposing medications, including anti-inflammatory, antiviral and immunomodulatory drugs, Figure 1. However, whether this strategy is efficient and effectual has yet to be established.

Empirical reports into the effects of H_2 as an Asclepius gas describe marked reductions in the levels of circulating pro-inflammatory cytokines, reduced M1 activation, neutrophil infiltration, and decreased DNA damage due to oxidation after application.[56-58] Such qualities make H_2 an ideal therapeutic for targeting dysfunctional intracellular processes that occur during infection, such as metabolic regulation and redox homeostasis, irregularities that are deemed significant to both pathogenesis and progression of COVID-19. In clinical studies, oxy-hydrogen inhalation therapies containing 66% H_2 and 33% O_2, delivered at 6L/min via nasal cannula, have been demonstrated to reduce chest pain and severe dyspnoea, improving the prognosis for nosocomial COVID-19 patients ($n = 90$).[47] The authors analyse the effects of 7-8 hours of daily oxy-hydrogen inhalation, comparing it with standard care O_2 therapy. The results show that O_2 saturation was significantly improved in patients who had oxy-hydrogen treatment ($p = <0.01$), and that severity of coughing and chest pain were appreciably reduced after 2-days of treatment.[47] Similarly, a retrospective study of the medical records of COVID-19 patients ($n = 24$), characterises a significant reduction in neutrophilia alongside depleted levels of C-reactive protein (CRP).[59] This analysis also determined that oxy-hydrogen was as effective as standard O_2 therapy in reducing elevated levels of cardiac distress markers (e.g., lactate dehydrogenase (LDH) and creatinine kinase (CK)) and biomarkers of hepatic damage (e.g., alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)), concluding that oxy-hydrogen protects against hypoxia-induced tissue damage through suppression of aberrant immune responses.[59] In further support of these findings, an assessment of oxy-hydrogen inhalation in patients with acute exacerbations due to chronic obstructive pulmonary disease (COPD), ($n = 108$) describes substantial reductions in cough, dyspnoea and sputum, and fewer adverse events after 7-days of treatment (3L/min/6-8 hours/day).[46] As H_2 inhalation therapies can be delivered by portable devices, needing only electricity and water to provide the gases, they can be readily deployed to remote and rural areas and used by untrained caregivers. Furthermore, the non-pressurised gas is created on demand, thereby reducing the risk to patient safety and negating the requirement for both secure storage facilities and specialised training. Although the clinical data for H_2 treatment of COVID-19 to-date is vestigial, further clinical[18-21] and experient evaluations[22-24,60-62] shall provide valuable insights into the impact of H_2 therapies on the corollary of acute COVID-19.

H_2 as an Anti-inflammatory Agent in the treatment of Acute COVID-19

Despite the precise molecular mechanisms behind H_2 activity being undiscovered, the antioxidant influence[15,63,64] of H_2 therapeutics is of fundamental importance when considering the aetiology of inflammatory-related conditions such as COVID-19. It is now well accepted that aberrant ROS/RNS production can lead to an increase in the cellular stress response involving upregulated expression of pro-inflammatory chemokines and cytokines, alongside scopic expression of adhesion molecules able to regulate the recruitment of monocytes and neutrophils from the circulation to sites of inflammation. During infection, the inflammatory response is initiated via toll-like receptor (TLR) recognition of danger- and, or, pathogen-associated molecular patterns (DAMPS and PAMPS, accordingly), with TLR hyperstimulation being the principal mechanism behind immunopathologies that can occur post-viral infection. DAMPS AND PAMPS are small molecules released during cellular injury (DAMPS) as a result of internal factors such as OxS and energetic dysfunction; or antigens of bacterial or viral origin (PAMPS).[65]

In response to infection cells begin to produce and release pro-inflammatory molecules. When such proteins and peptides are released into the bloodstream, they often lead to damage of the cells. Such assault on the cellular epithelium, for example, increases permeability of the vessels, which in turn, leads to increased chemical signalling and migration of white blood cells (WBC), perpetuating both OxS and the inflammatory cascade.[66] The formation of a pro-inflammatory milieu has broader influences on innate immunity through holistic signalling and the production and release of complement proteins. Activation of the complement cascade has multiple physiological effects including anti-viral activity, inducing high fever, promoting phagocytosis and attracting WBC which migrate to the site of infection.[67] Administration of H_2 has been demonstrated to downregulate genetic expression of pro-inflammatory cytokines,[68] inhibit mast cell activation,[68] enhance macrophage phagocytosis,[69] and restore levels of regulatory T-cells.[24] This combination is highly effective in reducing both the localised and systemic inflammatory responses that contribute to the rapid onset of inflammation during moderate and severe COVID-19.

It is now well documented that H_2 is a highly effective and practical-to-use anti-inflammatory agent and evidence suggests that H_2 inhalation can reduce biochemical markers of oxidative stress and pro-inflammatory peptides.[24-26,63-65,70-72] Two disparate methods have confirmed this theory in rodent models of disease. The first method examined the anatomical composition of tissues with histological staining; whilst the second focused on the direct measurement of oxidative biomarkers.[73] These results have significance when considering inflammatory-related respiratory conditions including, asthma, COPD and COVID-19. To illustrate, a clear reduction in the expression and serum concentrations of such constitutive pro-inflammatory mediators as C-reactive protein (CRP), interferon gamma (IFγ), tumour necrosis factor alpha (TNFα), and interleukins (e.g., IL-1β, IL-6) are noted when H_2-containing therapies are administered.[74,75] Recent reports into the effects of H_2 as a medical gas describe the significant reduction in pro-inflammatory cytokine production, reduced neutrophil infiltration and activity, and decreased oxidative damage to DNA, after administration.[76] These protective mechanisms are accomplished not only through interaction with multiple cellular processes, as described above, but also through regulating the activation of p38/MAPK signalling pathway. p38/MAPK is a fundamental protein complex responsible for initiating the biosynthesis of pro-inflammatory cytokines and therefore controlled inhibition of this pathway during COVID-19 by H_2 is likely to prevent hyperinflammatory responses and aide recovery.

The influence of H_2 as an immunomodulator extends beyond the p38/MAPK signalling cascade, increasing the activity and expression of nuclear factor erythroid 2–related factor 2 (Nrf2),[77] a transcription factor responsible for upregulating the production of endogenous antioxidants, whilst simultaneously downregulating expression profiles of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).[78] NF-κB, a transcription factor which is upregulated as a result of OxS, is responsible for promoting the genetic transcription of pro-inflammatory molecules and regulating inflammatory lymphocyte differentiation. Furthermore, enhanced levels of Nrf2 are known to amplify expression of haem oxygenase-1 (HO-1), a haem degrading enzyme that has a pivotal role in both reducing and preventing vascular inflammation.[79] Therefore, as well as modulating the immunological response via its influence on cytoprotective gene expression, Nrf2 also inhibits pro-inflammatory responses. Through enhancing the cellular availability of Nrf2, H_2 is likely to have a significant effect on the inflammatory profile during moderate-severe acute COVID-19.

The Aftermath of Acute Infection - Post-COVID-19 Syndrome

Post-COVID-19 syndrome is defined as continuous, relapsing or remittance of symptoms >28 days after infection and can be mild, moderate or severe in nature.[8,80,81] The corollary of a hyperactive immune response and persistent inflammation, infection-induced tissue damage, and stress related to the concomitant socioeconomic impact of the pandemic, are all likely to predispose individuals to post-COVID-19 syndrome. However, due to resolution of viral infection and the lack of serological antibodies, accurate diagnosis of this condition is complex. Common symptomatic manifestations are described as chronic fatigue, reported in 58% of cases, dyspnoea (36%), cognitive deficits (28%) and myalgia (26.5%).[8,27,28,80] Existent data suggests that up to 63% of patients diagnosed with post-COVID-19 syndrome experience symptoms that reduce their ability to undertake daily responsibilities,[82] whilst 17.8% of those in work before having COVID-19 were no longer employed. A reduced efficiency to perform routine tasks was noted to be more typical in nosocomial patients than in outpatients.[83] In children, the symptoms of post-COVID-19 syndrome develop despite the initial presentation of mild symptoms. Collated research indicates that approximately 10% of 2–11-year-olds have one or more persistent symptoms of COVID-19 35-days post-diagnosis, a figure that rises to 13% in adolescent 12–16-year-olds.[82]

Due the hyperactive and systemic inflammatory response experienced during acute infection, COVID-19 can contribute to major organ dysfunction and continued immune dysregulation present in post-COVID-19 syndrome. The prevailing symptoms of post-COVID-19 syndrome are documented as cardio-respiratory dysfunction, chronic fatigue and cognitive deficits.[84] Evidence suggests that fatigue is the most common symptom experienced with more than 50% of reported cases listing chronic, and, or exertive fatigue as symptoms, with research suggesting that persistent mitochondrial dysfunction, OxS and inflammation are synonymous with this condition.[85] Myalgic encephalomyelitis chronic fatigue syndrome (ME-CFS) is a highly individualised disorder which can present with a myriad of symptoms including cardiovascular distress (i.e., heart palpitations and irregular heartbeat),[85] cognitive dysfunction (e.g., anxiety, confusion, decreased clarity of thought, forgetfulness),[86] dizziness, and extreme tiredness.[87]

Molecular Hydrogen (H_2) as a Treatment for Post-COVID-19 Syndrome

Controlling levels of OxS and hyperactive immune responses during acute infection may be key to reducing morbidity and mortality as a result of COVID-19 and may also mitigate the progression into post-COVID-19 syndrome. Perhaps of pertinence here is that fatigue, cardiorespiratory dysfunction and cognitive anomalisms are also recognised consequences of such viral infections as Epstein-Barr (EBV), Influenza, Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) and SARS-CoV.[10] Therefore, extending research into the long-term effects of such infections is likely to provide valuable insights into the pathological progression disease, informing the rationale behind new treatment options.

As discussed above, H_2 is deemed to be highly effective as an anti-inflammatory and antioxidant compound, aspects that are significant to post-COVID-19 recovery as heightened ROS/RNS levels and protracted low-grade inflammation are considered to be notable contributary factors to both acute and chronic fatigue associated with post-COVID-19 syndrome.[72,88] Recent research into the effects of H_2 as a treatment for ME-CFS describes a superior reduction in symptoms of fatigue when compared with other nutraceuticals, including nicotinamide adenine dinucleotide (NADH) and Coenzyme Q (CoQ).[89] Collating data available from clinical resources, the authors note significant reductions in the physiological indices of fatigue, which include blood lactate levels, cyclic adenosine monophosphate (cAMP) signalling, and markers of oxidation (e.g., 8-Oxo-2'-deoxyguanosine). In addition, incidence of the psychological perception of fatigue were also greatly decreased after receiving treatment with H_2 products.[89] In further support of these findings, a pilot study investigating the effects of HRW on inflammation and fatigue in patients with mild-moderate COVID-19 symptoms established that consuming 1.5 litres of HRW containing 8 parts per million (0.4mM) H_2, each day for 14 days, resulted in a significant decline of patient reported fatigue, which was accompanied by a decrease in respiratory effort and improvement in O_2 saturation levels.[90] In addition to the cytoprotective action within the central nervous system, H_2 is identified as having beneficial effects for muscular fatigue,[4] often experienced by individuals recovering from COVID-19. Here, H_2 is shown not only to remediate oxidative stress and the damage caused by an excess of reactive gases, but also to reduce both genetic expression and serum levels of pro-inflammatory cytokines (e.g.,

interleukins, tumour necrosis factor-alpha), and chemokines (e.g., interferon gamma),[4] an aspect that promotes physical recovery as demonstrated in the aforementioned text. Furthermore, imbibition of HRW has been demonstrated to improve O_2 saturation and fatigue in a case study of post-COVID-19-like syndrome.[91] However, these results were not corroborated in a larger clinically controlled group[92] where almost doubling the concentration and duration of H_2 exposure (227mL of HRW, containing 1.6mg/L (0.8mM) of H_2, consumed up to five times a day for 28 days) did not produce significant data. In accordance, further in-depth research will be required if accurate dosage and treatment protocols are to be delineated.

SARS-CoV-2 infection can also directly affect the heart tissue, either via cardiac inflammation (myocarditis) or through stress-related cardiomyopathy, both of which impair optimally functionality of the heart.[93] Dysregulation of redox homeostasis is also known to augment inflammation within the cardiorespiratory and vascular systems[94] and is therefore highly likely to influence the pathology that limits cardiorespiratory and cognitive recovery in post-COVID-19 patients. Application of H_2 can reduce the cardiotoxicity that results from an increase in ROS/RNS production and heightened inflammatory responses.[95,96] Studies into the effect of H_2 during myocardial infarction[97] and post-cardiac arrest syndrome[98,99] detail favourable prognosis in H_2 groups, with the anti-inflammatory and antioxidant properties of H_2 being mooted as major contributors to these outcomes. As preliminary research into H_2 as a cardioprotective agent shows, the occurrence of cardiac injury and myocarditis in post-COVID-19 febrile paediatric patients has risen ten-fold since the pandemic began, with a recent study suggesting a link between SARS-CoV-2 infection and the increase in patients with multisystem inflammatory syndrome in children (MIS-C).[100] Here, SARS-CoV-2 infection can cause inflammation within the cardiorespiratory system, affecting both the blood vessels and pulmonary tissues and resulting in reduced O_2 exchange. The cardiac muscle then has to work harder to compensate for the reduced bioavailability of O_2, a situation which can cause lasting damage. Research regarding the cardioprotective effects of H_2 therapeutics consistently details marked reductions in vascular inflammation,[24,101] improvements in post-exercise heartrate recovery,[101,102] and enhancement of both physical and respiratory functions in acute-COVID[46] and post-COVID-19 patients.[62] As preliminary research into H_2 as a cardioprotective agent shows a favourable clinical profile, H_2 could be implemented into future treatment strategies for children and adults with long-lasting COVID-19 symptoms.

In relation to the neurological symptoms associated with post-COVID-19 syndrome, by targeting the underlying mechanisms behind neuronal dysfunction, namely inflammation and OxS, H_2 therapies have been demonstrated to mitigate long- and short-term memory loss in rodent models of systemic infection.[103] Building on this empirical research, observational studies of patients with Alzheimer's Disease demonstrate the potential of HRW to suppress OxS in APOE4 ascendant mild cognitive impairment.[104] Further to this, a randomised cross-over study on the effects of consuming 600mL of HRW containing 0.8-1.2 parts per million (0.4-0.6 mM) H_2, for four weeks showed that sympathetic nerve activity during the resting state was significantly reduced, suggesting that HRW may support homeostatic function in parts of the central nervous system that control temperament, anxiety levels, and autonomic nerve function.[105] In laboratory models, consumption of H_2 was demonstrated to positively affect neurons in the hippocampus, effectively reducing learning impairments and memory loss through decreased expression of pro-apoptotic proteins (e.g., caspase-3).[40] However, as mild cognitive impairment is an emerging symptom of post-COVID-19 syndrome, specific studies assessing the efficacy of H_2 to ameliorate COVID-19-related cerebral deterioration are warranted.

Future Perspectives

As H_2 is most sustainably produced through the electrolysis of water, utilisation within healthcare could be easily adopted with devices that produce either pure H_2 or oxy-hydrogen gas that can be mixed with other gases during anaesthesia, or directly inhaled via cannula delivery.[106] H_2 is diffusible in aqueous solutions and can be administered as HRW for ingestion and topical applications, or as HRS for use intravenously.[106] Research to date has demonstrated that H_2 can provide clinically favourable results, particularly when inflammatory- and oxidative stress-related factors are associated with disease.[24,45-47,60-62,95-101] However, knowledge gained from extensive clinical and empirical data has been slow to translate into global healthcare practices. Whether the reluctance to implement H_2 therapies is due to insufficient analysis of dosage and procedure protocols; or because the primary mechanism/s of action have

yet to be elucidated, is of cardinal interest if the drive towards sustainable and effective anti-inflammatory medications is to be endorsed by governing health institutions. For H_2 to be recognised as more than a food and lifestyle supplement it will be necessary to implement large, phase IV, clinical trials that identify effective strategies for treating numerous diseases.

Summary and Conclusion

To summarise, due to the constantly evolving nature of SARS-CoV-2 and the effect that has on COVID-19 associated illness, it is likely that current prophylaxis and treatment strategies will need to be revised if healthcare professionals are to diagnose and effectively treat such a challenging condition. H_2 is a novel and natural anti-inflammatory and antioxidant compound that, due to its low molecular weight, and polar and electrochemical neutrality, is able to rapidly traverse physiological barriers into organised tissue structures. Here, H_2 can diffuse beyond cellular and sub-cellular membranes, and effect such fundamental cellular processes as redox signalling and inflammatory responses. Such factors are relevant in view of the disease caused by SARS-CoV-2 infection, COVID-19. Acute COVID-19 illness is associated with a rapid increase in OxS as a result of viral incursion and release of prodigious amounts of inflammatory factors that can contribute to capacious tissue damage with long-lasting repercussions for patients. Through moderation of the redox status of the cell, studies have shown H_2 can mitigate damage caused by OxS[58,77] and modulate the expression of pro-inflammatory molecules.[57,58,60]

In conclusion, critical evaluation of recent studies strongly suggests that H_2-containing therapeutics such as oxy-hydrogen inhalation and HRW, for example, can reduce disease severity in acute COVID-19 and improve such parameters of post-COVID syndrome as chronic fatigue, inhibition of cardiovascular function and mild cognitive impairment. If, however, H_2 therapies are to be recommended as alternative or ancillary options for the treatment of COVID-19 illness, a comprehensive strategy involving clinical evidence, cost-benefit analysis, dosage concentrations and durations, and further mechanistic studies will be necessary.

Author Contributions

G.R. is responsible for the conceptualisation, graphical content and original draft preparation. A.N., A.T., and J.T.H., contributed to the draft manuscript and aided in the editing of the work. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

A. Nenov is a board member of Water Fuel Engineering. The remaining authors have declared no conflict of interest.

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