

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

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20 **Short Title: Hydrogen treatment for COVID-19**

21

22 **Abbreviations:**

23 **ACE2** angiotensin-2 converting enzyme, **ARDS** acute respiratory distress syndrome, **ATP** adenosine

24 triphosphate, **BALF** bronchial alveolar lavage fluid, **CAT** catalase, **COPD** chronic obstructive

25 pulmonary disorder, **COVID** corona viridae infectious disease, **DUOX** dual oxidase, **GAPDH**

26 glyceraldehyde-3-phosphate dehydrogenase **GM-CSF** granular macrophage colony stimulating factor,

27 **GSH** glutathione, **GST** glutathione S transferase, **HBV** Hepatitis B virus, **HRW** hydrogen-rich water,

28 **IF** interferon, **IL** interleukin, **MDA** malondialdehyde, **MAPK** mitogen-activated protein kinase,

29 **MERS** Middle-Eastern respiratory syndrome, **MPO** myeloperoxidase, **MODS** multiple organ

30 dysfunction syndrome, **NADH** nicotinamide adenine dinucleotide, **NADPH** nicotinamide adenine

31 dinucleotide phosphate, **NFκB** nuclear factor kappa-light-chain-enhancer of activated B cells, **NRF2**

32 Nuclear factor erythroid 2-related factor 2, **NOX** NADPH oxidase, **ORF** open reading frame, **OS**

33 oxidative stress, **ROS** reactive oxygen species, **RNS** reactive nitrogen species, **SARS** severe acute

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

36

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

39

40 **Abstract**

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

55

56 **Introduction**

57 H₂ is an uncharged, non-polar, diatomic molecule with a low molecular weight of 2.016 g/mol. These
58 characteristics make H₂ highly favorable for medicinal use as they allow H₂ to diffuse through both
59 the membrane of the cell and those that occur around organelles, such as mitochondria [1, 2]. The
60 distribution of H₂ across the membranes is not affected by electrochemical gradients and it can pass
61 through the hydrophobic phase of lipid bilayers. This allows molecular hydrogen to influence cytosolic
62 reactions and organelle biochemistry. These fundamental properties make H₂ an ideal therapeutic for
63 targeting dysfunctional intracellular processes including metabolic regulation and redox homeostasis.
64 Both of these biological factors that have been reported to contribute greatly to the pathological
65 progression of both infectious and non-infectious diseases [3].

66 Since the beginning of the 21st century, coronaviruses have caused two major global epidemics. In
67 2003, with a 10% lethal rate, severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1)
68 emerged as a unique and original human-tractable virus, which disseminated globally, reaching the
69 five densely populated continents of Africa, Asia, Europe and both the North and South Americas [4].
70 Additionally, in 2012 another contemporary and zoonotic coronavirus emerged in Saudi Arabia,
71 Middle East Respiratory Syndrome Coronavirus (MERS-CoV), a potentially fatal infection that also
72 proliferated to other countries across the world, and which has a mortality rate of 35% [5].

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

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

82 COVID-19 is known to present with variable symptoms that range from asymptomatic, yet contagious,
83 to obvious symptoms of respiratory infection with patients regularly presenting with a dry and
84 persistent cough accompanied by a fever in excess of 37.8°C. In some individuals these symptoms can
85 rapidly progress to respiratory failure due to alveola damage that necessitates assistance from a
86 mechanical breathing apparatus. COVID-19 can also lead to excessive production of inflammatory
87 biochemicals, which is referred to as a cytokine storm. It is proposed that H₂ therapy attenuates the
88 damage to alveola cells by remediation of destructive reactive oxygen/nitrogen species (ROS/RNS)
89 and through the reduction of excessive inflammation [7]. Undoubtedly one of the primary functions of
90 H₂ utilization in a clinical setting are the selective ROS antioxidant properties, first demonstrated by
91 Ohsawa *et al.* (2007) in a rodent model of ischemia/reperfusion injury [8]. Their results describe a
92 selective reduction of deleterious hydroxyl radicals ([•]OH) and peroxynitrite (ONOO⁻) molecules, but
93 no reduction in the important signaling molecules hydrogen peroxide (H₂O₂) and nitric oxide (NO[•])
94 [8]. Correspondingly, it is proposed that due the ability to diffuse through biological membranes
95 coupled with both anti-inflammatory and selective antioxidant properties, H₂ may dampen the effects
96 of the damaging cytokine storm [9], that underlies acute and severe SARS-CoV-2 symptomology.
97 Currently, however, treatment strategies vary between countries, and a consensus on a globally
98 accepted and definitive treatment plan has yet to be agreed upon. Nevertheless, The National Health
99 Commission of the People's Republic of China have provided a detailed intervention strategy that
100 includes administration of various antiviral medicaments (e.g. lopinavir, ritonavir, chloroquine
101 phosphate) along with inhalation of molecular hydrogen (66:33%; H₂:O₂) as an adjunctive therapeutic
102 that is able to be delivered through nasal cannulas [10].

103

104

105 **Aims**

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

109

110 **COVID-19: ‘Halo’ to a New Contagion**

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

118 Intense and rapid study of this new contagion has revealed SARS-CoV-2 to be an enveloped virus with
119 an outer adhesive coating of glycoproteins that form a corona, or halo shape when visualized using
120 transmission electron microscopy (Figure 1). Individual corona viruses are currently categorized into
121 4 classes, α , β , γ and δ . Contemporary reports suggest that α and β strains infect mammalian hosts,
122 while γ and δ are currently thought to only infect avian species [13]. Outbreaks of such viruses as
123 SARS (GenBank Accession: AY278488.2) (2003/4) and MERS (GenBank Accession: NC_019843.3)
124 (2015), have demonstrated that in particular β -coronaviruses have the ability to transfer between
125 species [14]. To date, only six coronaviruses are known to cause disease in humans, four (α -HCoV-
126 229E, α -HCoV-NL63, β -CoV-HKU1 and β -HCoV-OC43) are known to cause typically surmountable
127 and mild respiratory infections comparable to the common cold [15]. The other two viruses, SARS-
128 CoV-1 and MERS-CoV (with zoonotic transmission originating through bats and camels, respectively)

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

131

132 **Transmission, Incubation and Symptomology**

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

142 A study by Wang *et al.*, noted that the incubation period of COVID-19 ranged from 2-14 days [18].
143 Whilst a further study by Laur and associates [19] elucidated that the median incubation period was
144 estimated to be 5.1 days (95% CI, 4.5 to 5.8 days), with 97.5% of those developing symptoms within
145 11.5 days (CI, 8.2 to 15.6 days). Alarming, the range between showing symptoms to fatality has been
146 determined to be 6-41 days, with an average of 14 days [19]. However, it should be noted that these
147 figures are highly dependent on individual variation including the age and ethnicity of the patient as
148 well as their immune system status. To illustrate, the period between the onset of symptoms and severe
149 symptomology is shorter among the patients >70 years of age [7]. In other individuals, the infection
150 has been shown to present in various forms, ranging from an asymptomatic carrier state to pneumonia
151 and acute respiratory disease. Gastrointestinal symptoms such as diarrhea have also been noted in some
152 COVID-19 patients, a phenomenon also observed in patients infected with SARS-CoV-1 [20]. The
153 range of symptoms currently reported as the knowledge of the disease grows suggests a robust

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

161 Common symptoms that have been observed in patients with COVID-19 are associated with the
162 respiratory tract and frequently include a persistent, yet unproductive cough, dyspnoea, fever, and
163 pneumonia that can rapidly progress to severe and acute respiratory syndrome and even death [22].
164 Comparable to SARS-CoV-1 and MERS, SARS-CoV-2 is a novel β -coronavirus, that causes COVID-
165 19 respiratory disease that can further progress into acute respiratory distress syndrome (ARDS) in a
166 percentage of the population [23], especially the elderly, the immunocompromised, or those with
167 underlying health conditions such as pre-existing respiratory or metabolic disorders [24]. ARDS is a
168 common result of severe pulmonary infection involving Type II cell hyperplasia and membrane
169 permeability, jeopardizing the structural integrity of surfactant-producing alveolar cells [25].
170 Additional complications in those with severe COVID-19 have been noted to manifest within the
171 cardiovascular and renal systems that can progress into multiple organ dysfunction syndrome (MODS),
172 an immediate and life-threatening condition [12]. Differential identification of COVID-19 may involve
173 assessment of the oxygenation index, myeloperoxidase (MPO) activity, bronchial alveolar lavage fluid
174 (BALF) [26], all of which are parameters that undoubtedly increase the mortality rate for this particular
175 infection.

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177

178

179 **Epidemiology**

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

187 In Asia, the total number of cases reported to date is 875,625 among which 26,051 cases were fatal.
188 Active infection rates in Asia equals 343,504 and 4,941 of these are deemed critical cases. The South
189 American countries have totaled 521,321 cases, among these 26,779 fatalities have been attributed to
190 COVID-19 infection. 300,751 active infections have also been described in the South Americas, with
191 10,583 requiring critical care. In Africa, the total number of cases are 97,905 with 3,022 deaths, 55,260
192 active infections with only 288 of these viewed to be serious. Oceania total cases had reached 8,688
193 with 121 deaths, 542 patients with ongoing symptoms, with 10 of these receiving critical care [27].
194 This equates to an approximate global death rate of 4.62% of confirmed COVID-19 cases with a
195 marked increase noticed in Europe (9.01%) and North America (56.13%), when compared with Africa
196 (3.09%) or Asia (2.97%).

197

198 **Genomic Identification**

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

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

218 Scrutiny of the SARS-CoV-2 genome reveals a 79% sequence homology with SARS-Cov-1 and a 50%
219 similarity with MERS [16], and whilst SARS-CoV-1 and SARS-CoV-2 utilize ACE2 receptors to gain
220 entry into the body, MERS-CoV shows a preference for dipeptidylpeptidase-4 [32]. This may become
221 a relevant factor when considering suitable pharmacological agents that effectively neutralize viral
222 adhesion and/or replication. Moreover, the comparable sequence of these viruses suggests that the
223 current human-associated coronavirus has mutated from the local bat colonies of China. Wu *et al.*,
224 (2020), however, report that although bats are considered a nutritional delicacy in Chinese culture, and
225 this could provide a possible route of transmission, these products were not available at the wet market
226 thought to be at the center of the current outbreak [33]. This is a cause for concern as protein alignment
227 sequences, alongside phylogenetic analysis [34], show that similar receptor proteins have been
228 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

254 when considering an increasing oxidative environment during COVID-19 infection is the increase of
255 GM-CSF that particularly induces the expression of CD14⁺ and CD18⁺ monocytes known to express
256 the biphasic cytokine interleukin-6 (IL-6) [39].
257 IL-6 expression becomes significant during COVID-19 infection as this particular cytokine is largely
258 responsible for mediation of the acute-phase immunological response, a defensive physiological
259 reaction involving an increasing body temperature in an attempt to eradicate the pathogen or cause of
260 disease, as well as the activation of inflammatory immune cells, predominantly neutrophils and
261 macrophages [40]. Moreover, inflammatory granulocytes are considered to be a major contributing
262 factor to the increasing oxidative environment by way of the oxidative burst produced by inducible
263 NADPH oxidase (iNOX4), enzymes within the leukocyte allowing the release of copious ROS/RNS
264 species in defense against pathogens. It should also be noted that dedicated enzymes such as nitric
265 oxide synthases (iNOS, nNOS, eNOS) and dual oxidase (DUOX1/2) also contribute to increasing
266 ROS/RNS during cellular stress events [41]. Intense and acute elevation of ROS/RNS not only causes
267 damage to cellular structures via initiation of free-radical cascades, breaching phospholipid membrane
268 integrity, but such an elevation of reactive electrophilic species can also be responsible for the
269 modification of essential metabolic proteins such as glyceraldehyde-3-phosphate dehydrogenase
270 (GAPDH). These actions frequently lead to cessation of enzymatic activity [42], and fluctuation or
271 disturbance to the essential energy-producing processes of the cell, which is vital in the body's defense
272 against pathogenic violation.

273 **Current Treatment Strategies**

274 There are no specific antiviral medications or developed vaccines that have been globally
275 recommended for the treatment of COVID-19 [12]. However multiple strategies are being
276 implemented worldwide, many of which include combinations of the drugs listed in Supplementary
277 Table 1. In addition to these antiviral therapies, nosocomial treatment of COVID-19 often involves the
278 requirement of O₂ therapy, typically delivered by nasal cannula for severe respiratory symptoms, or in

279 critical cases invasive and precarious intubation methods may also be necessary [25]. In such
280 conditions H₂ should be used in combination with other treatments such as Anakinra or Tocilizumab,
281 or in conjunction with O₂, considering H₂ is likely to be a complimentary, restorative molecule.
282 Equally, since H₂ is able to help modulate and regulate cytokines (i.e. production and consequence), it
283 would be fitting to be used in combination with such targeted immunomodulatory drugs.

284

285 **H₂ – And what it can do**

286 Atomic hydrogen is the first element in the periodic table and the lightest element in the universe. It is
287 a reactive free radical and as such can only exist on Earth in its molecular forms by combining with
288 other atoms, forming a variety of molecules and compounds. When the radical combines with another
289 hydrogen atom, it forms molecular hydrogen, a diatomic gas (H₂) with a molecular weight of 2.016
290 g/mol [43]. As a result of its low molecular weight, small size, and nonpolar nature, H₂ is highly
291 diffusible and is able to permeate through the blood/brain barrier, lipid membranes, cytosolic fluid,
292 and into the cellular organelles. These properties of H₂ are deemed profoundly favorable considering
293 conventional antioxidants lack these abilities, and are therefore, likely to be therapeutically less
294 effective [44]. The salutary qualities of H₂ were first recognized by Dole and colleagues (1975) who
295 demonstrated that hyperbaric hydrogen therapy, with a dosage of 2.5% O₂: 97.5% H₂ at 8 atmospheres
296 of pressure, can reduce squamous cell carcinoma in murine models [45]. However, it was not until
297 2007, when Ohsawa *et al.* [8] demonstrated that H₂ had a selective antioxidant effect, favoring
298 reduction of non-signaling ROS/RNS, that the molecular mechanisms of H₂ activity began to be
299 elucidated.

300 Ongoing research into this area has revealed that H₂ exhibits properties that activate nuclear factor
301 erythroid 2-related factor 2 (Nrf2), a protein known to induce genetic transcription by binding to the
302 antioxidant response element (ARE), a cis-acting enhancer sequence accountable for the consequential
303 expression of >200 cytoprotective peptides, proteins and enzymes [46]. Once released from its partner

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

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

329 transcription of growth factors. Significantly, H₂ has been noted to decrease protein levels of NFκB,
330 p65, TNFα, IL-1b, and suppress various MAPK cascades involving ERK1/2, JNK, p38, etc. inhibit
331 the activation of MAPKs cascades involving ERK, JNK, and p38 [53].

332 The inflammation process is an innate immune response that is possibly initiated and certainly agitated
333 by any cellular redox imbalance. The innate response involves not only the lymphocytes previously
334 mentioned in the contagion section above, but also the production and release of complement proteins,
335 pro-inflammatory cytokines, and IgM antibodies, all of which contribute to the rapid production of
336 ROS [53, 54]. An early study of Hepatitis B infection (HBV) by Xia *et al.* [55] demonstrated that
337 administration of 1200-1800 mL per day of hydrogen-rich water (HRW), over six continuous weeks,
338 reduced serum markers of oxidative stress (xanthine oxidase [XOD], malondialdehyde [MDA]) and
339 increased antioxidant status (glutathione *S*-transferase [GST], SOD) [55]. Supporting evidence also
340 details that inhalation of H₂ can specifically suppresses oxidative stress and inflammatory markers
341 (MDA, GSH and IL-1, IL-6, TNF-α, CRP, respectively) associated with liver resection, in which
342 ischemia reperfusion injury occurs [56]. These findings were confirmed both histologically and by
343 direct measurement of oxidative stress in porcine models of disease [56].

344 Of importance to the global crisis created by the emergence of COVID-19 however, are the mounting
345 reports that suggest application of H₂ in human respiratory diseases may modulate the acute and
346 nocuous cytokine storm. This effectively diminishes the counterproductive inflammatory response and
347 reduces parenchymal cell hyperplasia, which has been confirmed in multiple rodent models of ARDS,
348 chronic obstructive pulmonary disorder (COPD), asthma and ventilator-induced lung injury [57-59].
349 Using similar rodent models, H₂ therapy has also been demonstrated to decrease airway resistance,
350 reduce free radicals, and decrease dyspnoea [60]. Studies have further demonstrated that H₂ inhalation
351 can ameliorate mucus production and reduce expression of cytokines IL-1β, IL-5, IFγ, and TNF-α [39,
352 47], and as such, H₂ could theoretically provide partial alleviation of the respiratory symptoms
353 associated with hospitalization and mortality of COVID-19 patients.

354 As described in the previous section, excessive production of cytokines and chemokines are known to
355 contribute to the inflammatory process, an action that in turn negatively influences enzymatic function
356 in glycolytic pathways. Of relevance to the investigations involving the use of H₂ as an adjunctive
357 therapy for severe respiratory disease is the mounting body of evidence suggesting H₂ has properties
358 able to regulate many metabolic pathways [44, 61-63]. For example, Niu *et al.*, reported that H₂
359 reverses the biological switch induced by inflammation from oxidative phosphorylation (oxphos),
360 which produces substantial amounts of ATP within the mitochondria, to the less efficient but
361 accelerated production of ATP via glycolysis [64], a phenomenon frequently described during
362 infection and cellular stress events. Furthermore, metabolic dysregulation is known to provoke and
363 intensify inflammatory responses that when working synergistically can cause a reduction in oxygen
364 availability and subsequent hypoxia. The lack of biologically available O₂ further causes a shift from
365 aerobic respiration to anaerobic respiration, which is an important factor for cellular energetics when
366 fighting disease and viral infection [61,62]. Crucially, the conversion from oxphos to anaerobic
367 glycolysis causes a reduction in the available ATP for the essential synthesis and activation of
368 regulatory and defensive biological molecules, imperative in the defense against severe and acute
369 respiratory infections.

370 Of interest for wider pulmonary conditions, H₂ has been noted to protect against cigarette smoke-
371 induced pulmonary emphysema in mouse models [57]. Here H₂ ingestion was shown to reduce DNA
372 damage, determined by a decrease in biological markers such as phosphorylated histone (H2AX) and
373 8-hydroxy-2-deoxyguanosine (8OHdG) and lower markers of senescence (e.g. β-galactosidase, p16
374 and p21), resulting in restored lung compliance [57]. Further studies into the consumption of HRW
375 have delineated that molecular hydrogen increases the elimination of fine carbon particles from the
376 lungs, thereby inhibiting lipid peroxidation of cellular membranes and attenuating lung injury [65].
377 Additionally, Terasaki *et al.*, noted that consumption of HRW alongside inhalation of H₂ protects

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

380

381 **Proposed Delivery Mechanisms of H₂**

382

383 Potentially, there are three routes for hydrogen administration in a clinical setting, ingestion of
384 HRW, inhalation of H₂ gas, and intravenous infusion of hydrogen-rich saline (HRS). Of these
385 possible application methods, infusion with HRS has been identified as having salubrious effects for
386 numerous lung conditions including acute lung injury (ALI) and ARDS [67, 68], both of which are
387 common in patients with severe COVID-19 infection [69]. Of particular interest are the
388 investigations that demonstrate that H₂ is an anti-apoptotic, anti-inflammatory, anti-oxidative agent
389 [2, 3]. Many of these reports describe significant reduction in proinflammatory cytokine production,
390 reduced neutrophil infiltration and decreased DNA oxidation. This is accomplished through multiple
391 mechanisms including the regulation of p38/MAPK and Bim/Bax activation that regulate
392 inflammatory and apoptotic processes, respectively [68, 69]. Also of interest when analyzing the
393 effects of H₂ on pulmonary disorders are the extensive reports that evidence induction of the
394 cytoprotective P13K/Akt pathway that is responsible for the downstream production of important
395 proteins such as claudin-5 protein, an adhesion molecule that protects against endothelial
396 permeability, and aquaporins 1 and 5 that have an essential role in the evacuation of accumulative
397 H₂O in ALI and ARDS [70-74].

398 Alternatively, hydrogen-rich water can be produced by infusing pure H₂ gas into water or beverages,
399 or via electrolysis. It is also routinely formed by the reaction between non-ionic metallic magnesium
400 (Mg) and H₂O, creating both magnesium hydroxide and molecular hydrogen according to the
401 equation: $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$. Commercially there are various hydrogen products on the
402 market ranging from portable tablets based on the magnesium reaction, which can deliver over 7
403 mg/L of H₂ [74], to ready-to-drink H₂ infused beverages [75]. There are also hydrogen water

404 machine technologies that can be large and static or incorporated into multiuse, small and portable
405 bottles. However, customers should be aware that many products may either not perform as
406 advertised and/or not provide doses of H₂ that are similar to what is used in clinical studies.
407 Lastly, another method of administration is inhalation of H₂ gas, which allows conveyance directly
408 into the pulmonary parenchyma and which can also be easily combined with O₂ therapy in an oxy-
409 hydrogen mixture. Here, gaseous H₂ can be created either by utilizing technology that employs a
410 proton exchange membrane requiring distilled water, or by way of electrolysis of H₂O enriched with
411 electrolytes such as sodium/potassium hydroxide or bicarbonate. Alternatively, tanks of pre-mixed
412 medical grade air containing 2-4% H₂, 21% O₂ and balance nitrogen are also used [76]. Regardless
413 of the method, the gases can be delivered by way of a nasal cannula or facemask. This approach may
414 allow clinicians to effectively administer both the oxygen needed to ensure optimal gas exchange
415 within the lungs whilst also limiting the likelihood of hyperoxia-induced ROS/RNS accumulation via
416 the aforementioned cytoprotective mechanisms. Hyperoxia is a condition that is known to intensify
417 bronchopulmonary dysplasia in the epithelium, and of significance for COVID-19 patients, in type II
418 alveolar cells [77]. Inhalation of H₂, either in conjunction with O₂, or through exclusive H₂
419 application, exhibits numerous cytoprotective qualities including upregulated expression of HO-1,
420 Nrf2 and antioxidative compounds (e.g. SOD, CAT), activation of P13K/Akt downstream events,
421 and inhibition of proinflammatory pathways involving NFκB, TNFα and IL-1β [77-79]. In a model
422 of ventilator-induced lung injury Huang *et al.*, described a potential hormetic effect to H₂ gaseous
423 employment, wherein H₂ application significantly reduced epithelial apoptosis by way of an initial
424 enhancement of NFκB availability in the first hour post-administration; however, when tested two
425 hours post-administration, NFκB levels were significantly reduced along with decreased levels of
426 pro-apoptotic markers (e.g. BAX) and edema [79].

427

428 **Conclusion and Recommendations**

429 Although the primary targets of H₂ remain elusive, there is a rapidly expanding body of evidence
430 detailing both the safety and efficacy of hydrogen treatment for numerous conditions. Of particular
431 significance, due to the emergence of the novel SARS-CoV-2, are the reports that H₂ may have a
432 salubrious effect in models of pulmonary inflammatory diseases, and those that detail abatement of
433 the pernicious cytokine storm, conceivably through the reduction of deleterious ROS/RNS (e.g. ·OH,
434 ONOO⁻) and regulation of multiple metabolic pathways. These are important factors in the
435 pathogenesis of COVID-19, and amelioration of the redox environment may reduce the onset of severe
436 symptomology in vulnerable patients.

437 Consequently, application of H₂, shown to be innocuous in anaesthetic doses, may provide an effective
438 adjunctive medicament to O₂ inhalation in the treatment of COVID-19, for the critically ill. Although
439 this method is recommended and practiced in the People's Republic of China [10] with
440 oxygen/hydrogen mixed gas noted to significantly reduce dyspnoea [80], it is not widely used
441 elsewhere. To date, only one clinical trial using oxyhydrogen for the treatment of COVID-19 infection
442 has been registered with the US National Library of Medicine, with a further four clinical trials
443 registered with The Centre for Evidence-Based Medicine (CEBM) [81]. Therefore, in the light of this
444 unprecedented global pandemic, it would be prudent to further investigate the effect of molecular
445 hydrogen, not only for the immediate threat of viral contagion, but also for other inflammatory
446 respiratory conditions such as pneumonia, asthma, COPD and cystic fibrosis. As H₂ has also been
447 demonstrated to work synergistically with NO^{*} [75] in combatting ROS overload and granulocyte
448 infiltration during an animal model of cardiac infarction [82] it may be prudent to extend research into
449 the combined effect of hydrogen therapy and other iatrogenic gases.

450 It is the authors considered opinion that inhalation of H₂ would be more a more effective delivery
451 mechanism for patients with moderate/severe symptoms of COVID-19. Also worthy of notation is that
452 currently, most, but not all clinical trials have been based on inhalation of H₂, with this also being the
453 preferred delivery method as recommended by The National Health Commission of the People's

454 Republic of China [10]. However, in some cases, such as Parkinson's Disease, HRW has been
455 demonstrated as being more effective against disease progression [44]. Hence, the authors also suggest
456 multiple clinical studies are required to determine the best method of H₂ administration.
457 Future research into the utility of H₂ within the clinical setting may also wish to reflect on the
458 sustainability of hydrogen therapy with a view of employing this inexpensive and effective resource
459 as an ancillary treatment for multiple pulmonary disorders.

460

461 **CONFLICT OF INTEREST STATEMENT**

462 The author confirms that they have no competing interests.

463

464 **ACKNOWLEDGEMENTS**

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

467

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712

713 Tables

714 Table 1.

715

VIRUS CLASSIFICATION	
REALM	<i>Riboviria</i>
PHYLUM	<i>Incertae sedis</i>
ORDER	<i>Nidovirales</i>
FAMILY	<i>Coronaviridae</i>
GENUS	<i>Betacoronavirus</i>
SUBGENUS	<i>Sarbecovirus</i>
SPECIES	<i>SARS-related coronavirus</i>
STRAIN	<i>SARS-CoV-2</i>

716

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

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719 Figures and Legends:

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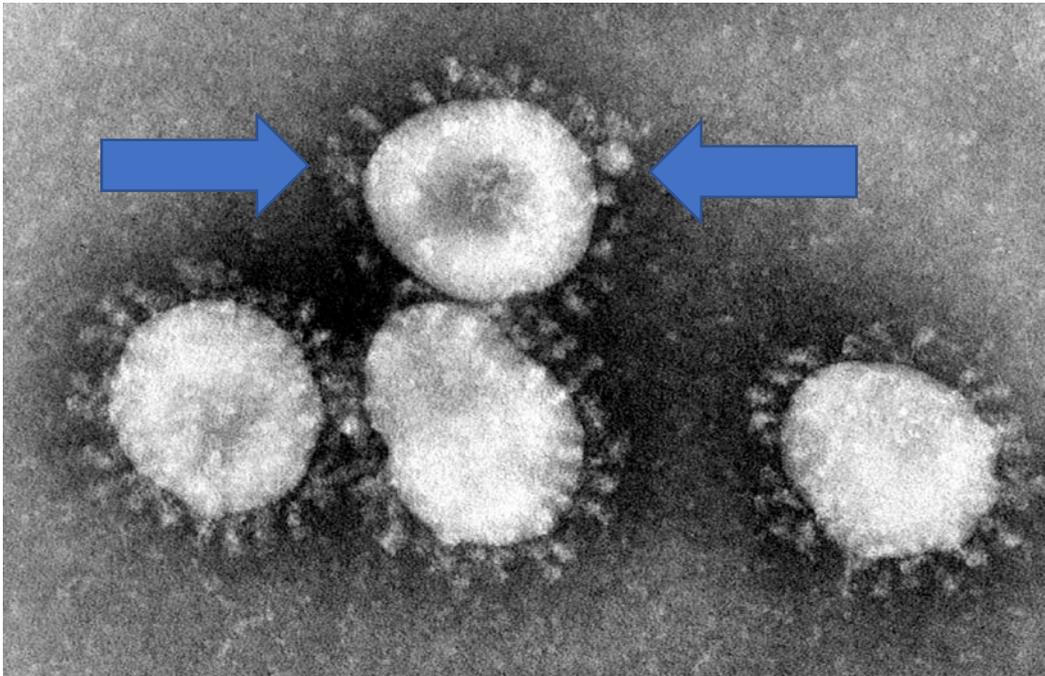


Figure 1: Electron micrograph of typical *corona viridae*. Blue arrow denotes the external glycoproteins that form a halo, or corona, around the capsid. Photo by Unknown Author is licensed under CC BY-SA Creative Commons.

Figure 2:

KEY:

- SARS-CoV-2 (COVID-19)
- SARS-CoV-1
- Bat - HKU3

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MFVFLVLLP----LVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFL 56
MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFL 60
MKILIFAFLANLAKAQEGCGIISRKPQPKMAQVSSRRGVYNDDEIFRSDVLHLLTQDYFL 60
* ::: . : . * : : . * ***** *.:***. *: *** **

PFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQS 116
PFYSNVTGFHTIN-----HTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNKSQS 113
PFDSNLTQYFSLNVDSDRYTYFDNPIIDFGDGVYFAATEKSNVIRGWIFGSSFDNTTQS 119
** ***: * :.: : * .***: * **:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*
760
LLIVNATNVVIKVFCEQFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSQPFL 176
VIIINNSTNVVIRACNFELCDNPFVAVSKPMG----TQTHMIFDFAFNCTFEYISDAFL 169
AVIVNNSTHIIIRVCNFNLCKEPMYTVSR--G----TQQNAWVYQSAFNCTYDRVEKSFQ 173
:*****:*****:*****: * . : . :. . * ***: : . . *

MDLEGKQGNFKNLREFVFNKIDGYFKIYSKHTPINLVRDLPPQGFSALEPLVDLPIGINIT 236
LDVSEKSGNFKHLREFVFNKIDGFLYVYKGYQPIDVVRDLPSGFNTLKPILKLPFGINIT 229
LDTTPKTGNFKDLREYVFNKRDGFLSVYQTYTAVNLRGLPTGFSVLKPIILKLPFGINIT 233
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770
RFQTLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPL 296
NFRAILTAFS-----PAQDIWGTSAAYVGYLKPITFMLKYDENGTITDAVDCSQNPL 283
SYRVVMAMFS-----QTTSNFLPESAAAYVGNLKYSTFMLRFNENGTITDAVDCSQNPL 287
:~::~ . : . : :***:** * : **:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

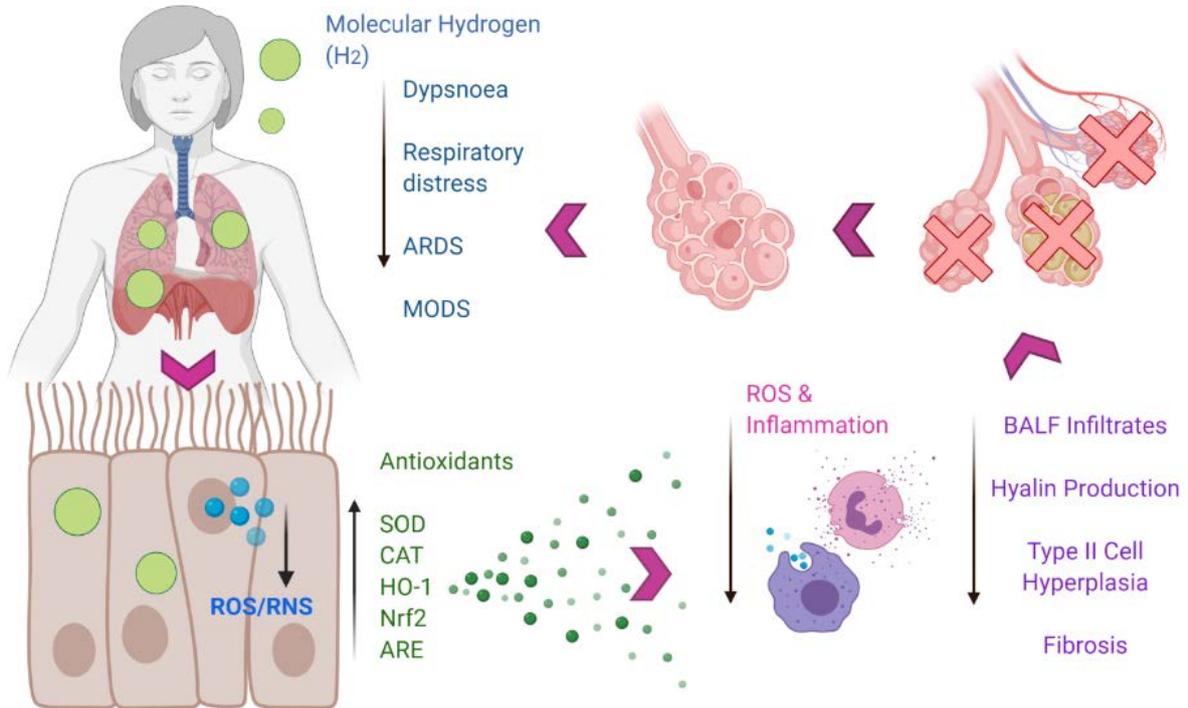
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871 **Figure 3a. Depicts cellular defense mechanisms triggered by SARS-CoV-2 infection that often**
872 **result in the requirement for nosocomial interventions**

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875 **Figure 3b**



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

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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-1 α and IL-1 β 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]

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

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