








Molecular Hydrogen Therapy For Management Of COVID-19

INTRODUCTION

Molecular hydrogen (H_2) has been proposed to have therapeutic and preventive effects for variety of diseases. Its applications range from acute illness such as ischaemia–reperfusion injury, shock and damage healing to chronic illness such as metabolic syndrome, rheumatoid arthritis and neurodegenerative diseases.¹ There is a growing evidence obtained by animal model experiments on molecular hydrogen (H_2) as antioxidant, anti-inflammatory, antiapoptotic and antiallergic.²⁻⁹ The claimed benefits were demonstrated through various delivery methods including drinking H_2 rich water, intra-peritoneal injection, infusion of H_2 -rich saline, taking an H_2 bath, dropping H_2 -saline onto the eyes and inhalation.(Table 1)^{5, 6, 10-15} However, inhalation of hydrogen gas has been established as the easiest and simplest route of administration. It also allows monitoring of the dose of hydrogen. As a biological gas, hydrogen has the ability to diffuse freely across biological membranes, acting in various functional capacities.^{16, 17}

Table 1: *In vivo* H_2 delivery systems^{14, 15}

Administration	Preparation/delivery method	Characteristics	Examples
Inhalation	Inhale gas mixture containing H_2 ($\leq 4\%$)	1)Rapid action, straightforward delivery 2)Does not influence blood physiological parameters (temperature, blood pressure, pH, pO_2) 3)Suitable to defense against acute oxidative stress 4)Unpractical to dose continuously	 <p style="text-align: center;">Inhalation</p>  <p style="text-align: center;">Aerosol inhalation</p>

Oral intake of hydrogen-rich water	Dissolving H ₂ in water up to 0.8 mM under atmospheric pressure at room temperature.	<p>1) Convenient, easily administered</p> <p>2) Easily evaporates and is lost in the stomach or intestine</p> <p>3) Difficult to control H₂ concentration administered</p>	 <p>Hydrogen-rich stick</p>
Injection of hydrogen-rich saline	<p>Intravenous injection</p> <p>Intraperitoneal injection</p> <p>Intrathecal injection</p> <p>Intravitreal injection</p>	Delivery of more accurate H ₂ concentrations	-
Direct incorporation	<p>Bath, mask</p> <p>Cold storage of transplanted organs</p> <p>Eye drops</p> <p>Spray on plants or immerse plants</p>	Convenient	 <p>Bath</p>  <p>Mask</p>
Increased intestinal hydrogen	<p>Oral drugs (e.g. acarbose, lactulose)</p> <p>Dietary (e.g. turmeric)</p>	Convenient	-

The molecular hydrogen is claimed to be beneficial in two ways in the ongoing epidemic of coronavirus disease 2019 (COVID-19);

1. As a therapeutic antioxidant

One of the major mechanisms the COVID-19 virus causes illness is by oxidative stress, producing breakdown products of oxygen including superoxide radical, hydrogen peroxide and hydroxyl radical, which are referred collectively as reactive oxygen species (ROS). These unstable radicals cause damage to various molecules in the body such as fats (lipid peroxidation and cell membrane damage), DNA (genetic malformations) and proteins (enzyme damage). In acute viral-

induced oxidative stress, this process is accelerated and may overwhelm the innate ROS detoxification system causing both cellular and organ damage and potential failure.^{18, 19} Molecular hydrogen eliminates free radicals by acting as specific scavenger of highly active oxidants, hydroxyl radical (OH) and peroxynitrite (ONOO). It also indirectly reduces oxidative stress by regulating the expression of various genes.^{14, 20}

2. As an anti-inflammation

Viral infection is capable of producing an excessive immune reaction in the host by stimulating massive release of cytokines. Unfortunately, at higher levels these same cytokines, in what is sometimes called 'cytokine storm', may cause increased inflammation in the tissues. Dysregulation of immune responses following hyper-inflammation and cytokine storm, may lead to multiple organ failure, pulmonary tissue damage (diffuse alveolar damage with inflammatory infiltration and oedema, interstitial fibrosis) and reduced lung capacity which is well-known in patients with COVID-19 infection.^{19, 20} Molecular hydrogen inhibits oxidative stress-induced inflammatory tissue damage via downregulation of pro-inflammatory and inflammatory cytokines.^{14, 20}

EVIDENCE on EFFECTIVENESS and SAFETY

Effectiveness

Following extensive search through available scientific databases (Ovid MEDLINE, Cochrane Database, PubMed) and Google search engine, a total of 16 articles were identified and included in this review. The articles comprise of one randomized control trial (RCT) and 15 animal studies on the effectiveness of molecular hydrogen therapy as anti-inflammatory and anti-oxidant in hypoxia/re-oxygenation induced lung injury as well as sepsis related conditions. These conditions exhibit similar mechanism of illness as COVID-19. All the studies were undertaken in China^{21-25, 27-36} except for one experimental animal study²⁶ which was conducted in Japan. Of the 16 included studies, eight studies (one RCT²¹ and seven experimental animal studies²²⁻²⁸) assessed the effect of hydrogen therapy on hypoxia/re-oxygenation induced lung injury caused by various lung conditions namely asthma (two studies)^{23, 24}, chronic obstructive pulmonary disease/ chronic injury induced by hypoxia/re-oxygenation (four studies)^{21, 22, 25, 28}, hyperoxic injury (one study)²⁶ and ventilator induced lung injury (one study)²⁷. The remaining eight studies examined the preventive and therapeutic applications of molecular hydrogen in the treatment of sepsis.²⁹⁻³⁶ There were three methods of molecular hydrogen therapy utilized in the experiments: inhalation of hydrogen (H₂) (11 studies)^{21-27, 29-32}, injection of hydrogen-saturated saline (four studies)^{28, 33-35} and oral intake of hydrogen-rich water (one study)³⁶. (Table 2)

Table 2: Evidence on molecular hydrogen as antioxidant and anti-inflammatory

First Author, Publication Year	Disease/pathological and physiological condition	Method of administration	Outcomes	Authors' conclusion
Human study				
	Respiratory System			
<p>Gong ZJ, 2016, RCT²¹</p> <p>(Double blinded controlled trial)</p>	<p>Chronic exposure to air pollutant-sanitation workers exposed to haze</p> <p>(96 sanitation workers)</p>	<p><u>Treatment group</u></p> <p>Inhaled H₂:O₂ mixture (66.67%:33.33%) 1 hour per day for 30 days</p> <p>[48 participants]</p> <p><u>Control group</u></p> <p>Inhaled N₂:O₂ mixture (66.67%:33.33%) 1 hour per day for 30 days</p> <p>[48 participants]</p>	<p>Protective effect to airway inflammation and oxidative stress</p> <p>-Improved cough symptom</p> <p><u>Lung function test</u></p> <p>-Higher FEV₁ compared to control [(97±14)% vs (95±12)% (F=8.5, P<0.05) on 30th day of treatment]</p> <p>-Higher PEF compared to control [(73±15)% vs (67±18)% (F=8.68, P<0.05) on 15th day of treatment]</p> <p><u>Airway inflammation</u></p> <p>-Lower FeNO levels (marker for airway inflammation) in the treatment group [(16±5)×10⁹ vs (21±14)×10⁹ on 8th day of treatment, with significant difference (F=6.94, P<0.05)]</p> <p>-The sputum levels of MMP-12 (inflammatory marker) and SOD3 (oxidative stress marker) were consistently lower in the treatment group as compared to the control group</p>	<p>Inhalation of hydrogen gas could reduce airway inflammation and oxidative stress of the lungs exposed to air pollution.</p> <p>There was a significant inhibitory effect on the level of systemic inflammatory response.</p> <p>Inhalation of hydrogen could improve respiratory symptoms such as cough.</p>

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<i>Animal studies -Experimental studies</i>				
	Respiratory System			
Chen M, 2018 ²²	Chronic injury induced by hypoxia/re-oxygenation (H/R).	4% H ₂ inhalation	<u>Antioxidant</u> - Attenuated H/R-induced infiltration of inflammatory cells and alveolar wall thickening; reduce lung injury score (HR: 5.94 ± 1.96, HR+H ₂ :2.11 ± 0.38) - Reduced H/R-induced production of hydroxyl radicals (HR: 0.72 ± 0.07µM/mg, HR+H ₂ : 0.48 ± 0.02 µM/mg) <u>Anti-inflammatory</u> - Lowered the level of inflammatory markers (IL-1β HR: 2137 ± 63 pg/mg, HR+H ₂ : 1828 ± 124 pg/mg)(TNF-α HR:1032 ± 48 pg/mg, HR+H ₂ : 740 ± 85 pg/ml)	Molecular hydrogen improves H/R-induced lung injury by inhibiting hydroxyl radical production and inflammation in lungs
Huang P, 2019 ²³	Asthma	H ₂ inhalation (mixed gases 67% H ₂ and 33% O ₂) twice a day (2h once) for 7 consecutive days.	- Alveolar macrophages isolated from ovalbumin (OVA)-induced asthmatic mice showed decreased phagocytic capacity to Escherichia coli when compared with those of control mice. Defective phagocytosis in asthmatic mice was reversed by hydrogen gas inhalation.	Hydrogen gas inhalation enhanced alveolar macrophage phagocytosis in OVA-induced asthmatic mice, which may be associated with the antioxidant effects of hydrogen gas and the

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			<p>-Significantly alleviated OVA-induced airway hyperresponsiveness, inflammation and goblet cell hyperplasia, diminished TH2 response and decreased IL-4 as well as IgE levels, reduced malondialdehyde (MDA) production and increased SOD activity.</p> <p>-Inhibited NF-κB activation and markedly activated Nrf2 pathway in OVA-induced asthmatic mice.</p>	activation of the Nrf2 pathway.
Zhang N, 2018 ²⁴	Asthma	Inhalation-mixed gas consisting of 67% H ₂ and 33% O ₂ produced by the hydrogen oxygen nebulizer, for 60 min once a day for 7 consecutive days	<p><u>Decreased lung resistance</u></p> <p>- Lower RL in the treatment group compared with the control group (2.052 ± 1.2 cm/H₂O/ml/s vs 3.53 ± 1.9 cm/H₂O/ml/s, P < 0.05)</p> <p><u>Improved the mucus production</u></p> <p>- Reduced accumulation of inflammatory cells (eosinophils 2.22 ± 0.67 vs 3.22 ± 0.67, p < 0.01)</p> <p>- Reduced the epithelial goblet cell hyperplasia (2.9 ± 0.73 vs 4.00 ± 0.81, p < 0.01)</p>	Hydrogen gas inhalation improves lung function and protects established airway inflammation in the allergic asthmatic mice model which may be associated with the inhibition of oxidative stress process.

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			<p><u>Reduced the elevated levels of inflammatory cytokines</u></p> <p>IL-4 (18.91 ± 10.66 pg/ml vs 42.11± 24.31 pg/ml, p < 0.05), IL-13 (32.57 ± 4.43 pg/ml vs 68.04±35.26 pg/ml, p < 0.05), TNF-α (26.12 ± 5.59 pg/ml vs 38.62± 14.12 pg/ml, p<0.05) and CXCL15 (106.3±40.75 pg/ml vs 141.4 ± 40.75 pg/ ml, p< 0.05)</p> <p><u>Reduced the oxidative stress index presented in lung homogenates</u></p> <p>Reduction of MDA [1.82 (1.50–2.56) nmol/ mg vs 5.37 (3.85–6.59) nmol/mg, p < 0.05] and MPO levels [1.11 (0.89–1.31) U/g vs 1.51 (1.41–1.76) U/g, p < 0.05) was observed.</p>	
Liu X, 2017 ²⁵	Development of COPD-like lung disease in a cigarette smoke-induced rat model	Inhalation using hydrogen atomizer i) HI group - 21% O ₂ + 2% H ₂ by inhalation	- Reduced the number of inflammatory cells in the bronchoalveolar lavage fluid, and the mRNA and protein expression levels of tumor necrosis factor alpha, IL-6, IL-17, IL-23, MMP-12, caspase-3, and caspase-8, but increased the tissue inhibitor of metalloproteinase-1 expression.	Hydrogen inhalation slows the development of COPD-like lung disease in a cigarette smoke-induced rat model. Higher concentrations of hydrogen may represent a more effective way for the rat model.

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		<p>ii) Hm group - 21% O₂ + 22% H₂ and 15% O₂+85% N₂ at a flow rate of 1:2</p> <p>iii) Hh group - 21% O₂ + 41.6% H₂ and 100% N₂ at a flow rate of 5:3.</p> <p><u>Frequency</u>: once a day for 2 hours in a total of 4 months of cigarette exposure.</p>	<p>- Hydrogen inhalation improved lung pathology, lung function, and cardiovascular function and reduced the right ventricular hypertrophy index.</p> <p>- Inhalation of 22% and 41.6% hydrogen showed better outcome than inhalation of 2% hydrogen.</p>	
Kawamura T, 2013 ²⁶	Hyperoxic lung injury	2% H ₂ inhalation	<p>1) <u>Reduced lung dysfunction after hyperoxic exposure and prolonged survival against lethal hyperoxia in rats</u></p> <p>-all rats exposed to hyperoxia with 2% nitrogen died within 64 h, whereas rats exposed to hyperoxia with 2% hydrogen survived a median of 72 h (range 72–120 h)</p>	Hydrogen gas protected against hyperoxic lung injury both by decreasing the extent of oxidative injury caused by ROS, through hydrogen's free radical scavenging activities and by inducing Nrf2-dependent

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			<p>2) <u>Reduced lung permeability, lung edema, and alveolar-capillary leakage induced by hyperoxia</u></p> <p>-improved hyperoxia-induced lung edema, by reducing pleural effusion volume and by a significant decrease in the W/D ratio compared with lungs from hyperoxia/N₂ rats</p> <p>3) <u>Reduced hyperoxic lung injury and the expression of proinflammatory cytokines</u></p> <p>- both edema and inflammatory cell infiltration were reduced despite exposure to hyperoxia</p> <p>- treatment with 2% hydrogen during hyperoxic exposure significantly reduced the peak expression of the transcripts for the inflammatory mediators; the mRNAs for IL-1β, IL-6, TNF-α and ICAM-1</p> <p>4) <u>Modulated the Keap1/Nrf2 signaling pathway</u></p> <p>- significantly upregulated these Nrf2-dependent transcripts in rats exposed to hyperoxic conditions.</p>	protective signaling pathways.
Huang CS, 2010 ²⁷	Ventilator induced lung injury (VILI)	2% H ₂ inhalation	-H ₂ treatment inhibited upregulation of the mRNAs for pro-inflammatory mediators and induced antiapoptotic genes.	Inhaled hydrogen gas reduced VILI-associated inflammatory responses, at both a local and systemic level, via its antioxidant, anti-inflammatory and

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			<p>-In the lungs treated with hydrogen, there was less malondialdehyde compared with lungs treated with nitrogen.</p> <p>-Longer exposure to mechanical ventilation within lower tidal volume (10 mg/kg, 5 hours) caused lung injury including bronchial epithelial apoptosis. Hydrogen improved gas exchange and reduced VILI-induced apoptosis.</p>	antiapoptotic effects.
Liu Z, 2017 ²⁸	Tobacco smoke-induced COPD	<p>Hydrogen-rich saline (HRS)</p> <p>-10 ml/kg hydrogen-rich saline intraperitoneally for 4 weeks</p>	<p>-Decreased lung airway resistance and increased lung compliance and the ratio of forced expiratory volume in 0.1 s/forced vital capacity in chronic obstructive pulmonary disease rats</p> <p>-Lowered the levels of pro-inflammatory cytokines (IL-8 and IL-6) and anti-inflammatory cytokine IL-10 in bronchoalveolar lavage fluid and serum of chronic obstructive pulmonary disease rats.</p>	Administration of hydrogen-rich saline improves lung function and alleviates morphological impairments of lung through alleviating inflammation, reducing oxidative stress and lessening mucus hypersecretion in tobacco smoke-induced COPD rats

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	Severe infection/ Sepsis/ Septic Shock			
Liu W, 2013 ²⁹	Septic shock	2% H ₂ inhalation	Anti-inflammatory (IL-6, IL-8, TNF- α , and MPO) Antioxidant (\cdot OH, MDA, and SOD) Inhibition of apoptosis (Fas, Bcl-2)	Combined early fluid resuscitation and hydrogen inhalation may protect the lung and intestine of the septic shock rats from the damage induced by oxidative stress and the inflammatory reaction.
Xie K, 2010 ³⁰	Sepsis - zymosan (ZY)-induced generalized inflammation model	2% H ₂ inhalation - 2% H ₂ inhalation for 1 h and 6 h after zymosan injection	1)Anti-inflammatory (TNF- α , HMGB1) Antioxidant (SOD, 8-iso-PGF2 α) - decreased levels of oxidative product, increased activities of antioxidant enzyme, and reduced levels of early and late proinflammatory cytokines in serum and tissues. 2)Improved the 14-day survival rate of ZY-challenged mice from 10% to 70%	H ₂ treatment protects against multiple organ damages in ZY-induced generalized inflammation model, suggesting the potential use of H ₂ as a therapeutic agent in the therapy of conditions associated with inflammation-related multiple organ dysfunction syndrome.

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Xie K, 2010 ³¹	Sepsis	2% H ₂ gas -2% H ₂ inhalation for 1 h and 6 h after cecal ligation and puncture (CLP)	Anti-inflammatory (HMGB1, MPO) Antioxidant (CAT, SOD, and 8-iso-PGF ₂ α) - attenuated lung myeloperoxidase activity, wet-to-dry weight ratio, protein concentration in bronchoalveolar lavage, serum biochemical parameters - decreased levels of oxidative product, increased activities of antioxidant enzymes, and reduced levels of high-mobility group box 1 in serum and tissue	H ₂ inhalation may be an effective therapeutic strategy for patients with sepsis and sepsis-associated organ damage
Xie K, 2012 ³²	Septic shock -septic mice with moderate cecal ligation and puncture (CLP)	2% H ₂ and 98% hyperoxia -2% H ₂ and 98% hyperoxia for 3 h and 6 h after CLP	Anti-inflammatory (TNF-α, HMGB1, IL-10, and MPO) Antioxidant (CAT, SOD, and 8-iso-PGF ₂ α) -decreased levels of oxidative product (8-iso-prostaglandin F ₂ α), increased activities of antioxidant enzymes (superoxide dismutase and catalase) and anti-inflammatory cytokine (interleukin 10), and reduced levels of proinflammatory	Combination therapy with H ₂ and hyperoxia provides enhanced therapeutic efficacy via both antioxidant and anti-inflammatory mechanisms and might be potentially a clinically feasible approach for

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			cytokines (high-mobility group box 1 and tumor necrosis factor α) in serum and tissues	sepsis.
Tao B, 2016 ³³	Acute lung injury model (Septic rat lungs)	Hydrogen-rich saline (HRS) - intraperitoneal injection HRS (10 mL/kg) at 1 h and 4 h after the lipopolysaccharide LPS challenge (inducer for acute lung injury).	-Improved lung function -Inhibited LPS-induced activation of p38 mitogen-activated protein kinase and jun N-terminal kinase, which is associated with LPS-induced downregulation of AQP1 and AQP5 in the rat lung	HRS has protective effects against sepsis-related lung injury which occurred by preserving the expression of AQP1 and AQP5
Chen HG, 2013 ³⁴	Lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages	Incubation with hydrogen-rich saline	Anti-inflammatory (TNF- α , IL-1 β , HMGB1, and IL-10) Nrf2/HO-1 signaling pathway	Molecular hydrogen exerts a regulating role in the release of pro- and anti-inflammatory cytokines in LPS-stimulated macrophages, and this effect is at least partly mediated by HO-1 expression and activation.

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Li GM, 2013 ³⁵	Rat model of polymicrobial sepsis	Hydrogen-rich saline -5 mL/Kg hydrogen-rich saline at 0, 6, and 18 h after CLP	Anti-inflammatory (TNF- α , IL-6, HMGB1, IL-10, HMGB1, and MPO) Antioxidant (MDA, SOD) -reduced the serum high-mobility group box, alanine aminotransferase, creatinine, and blood urea nitrogen levels; the pulmonary interleukin 6, high-mobility group box, nitric oxide, and MDA levels - no significant difference in survival rate between the treatment group and control group	Hydrogen-rich saline has potential protective effects against sepsis by decreasing proinflammatory responses, oxidative stress, and apoptosis in a rat model of polymicrobial sepsis.
Zhang J, 2014 ³⁶	Acute peritonitis	Hydrogen rich water (3 ml per rat) was orally administered by gavage for 7 days beforehand and 3 days after 3 peritonitis modeling.	-Lower the levels of WBCs, plasma endotoxin and cytokines, enhance GSH activity, reduce MPO and MDA activities in the peritoneum tissue when compared with that of groups with only saline treated. -Decrease the NF- κ B expression in the peritoneum tissues.	Hydrogen-rich water could alleviate the severity of acute peritonitis, and it might perform this function by its anti-inflammation, anti-oxidation and anti-bacterial effects and reducing NF- κ B expression in the peritoneum tissues.

Biomarkers: MMP-12 = matrix metalloproteinases-12 (inflammatory marker); SOD3 = superoxide dismutase (oxidative stress marker); MDA = malondialdehyde; MPO = myeloperoxidase

Safety

The use of inhalational hydrogen gas among patients with tracheal stenosis in one experimental study, reported no adverse reaction or inhalation related discomfort occurred.³⁷

Safety is a primary concern with respect to H₂ transportation, storage and administration. In low concentration (4.1% in pure oxygen or 4.6% in the air), hydrogen is neither explosive nor dangerous. Previous preclinical studies highlighted explosive safety concern whereby flammable gas contained in the mixed gas cannot exceed one third of the lower explosion limit (4%) and these studies were able to administer a maximum dose of 2.9% hydrogen gas.³⁸⁻⁴⁰ Others thought it was safer to dissolve hydrogen into water and administer the hydrogen-rich saline by oral or by injection.⁴¹

CONCLUSION

There was very limited evidence on the effectiveness of molecular hydrogen therapy. Animal studies indicated this treatment may have protective and therapeutic effects in patients with lung injury and sepsis. More clinical trials are needed to prove the clinical safety of its use and the therapeutic effects of molecular hydrogen in human subjects.

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Based on available evidence up to 29 May 2020

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Disclaimer: This rapid assessment was prepared to provide urgent evidence-based input during COVID-19 pandemic. The report is prepared based on information available at the time of research and a limited literature. It is not a definitive statement on the safety, effectiveness or cost effectiveness of the health technology covered. Additionally, other relevant scientific findings may have been reported since completion of this report.

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