Emerging mechanisms and novel applications of hydrogen gas therapy

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Abstract

Clinical and pre-clinical studies have reported a broad range of applications for hydrogen gas therapy. Classically, conventional antioxidant therapy is limited because it neutralizes both the detrimental and protective effects of reactive oxygen species. As a weak reducing agent, hydrogen gas avoids this paradox by reacting with strong oxidants while leaving other beneficial oxidants reactive. This review gathers a promising list of hydrogen gas applications that merit further mechanistic investigation and additional therapeutic trials. Reports support the ability of hydrogen gas to downregulate the expression of pro-inflammatory cytokines and pro-apoptotic factors. Mechanistically, hydrogen gas has been shown to downregulate miR-9 and miR-21, while upregulating miR-199 to reduce inflammatory injury. In angiogenic pathways, hydrogen's inhibition of cyclic guanosine monophosphate-degrading phosphodiesterase led to higher levels of cyclic guanosine monophosphate, activation of protein kinase, and angiogenesis; next, as hydrogen gas increased the levels of intracellular calcium, stimulated vascular endothelial growth factor increased nitric oxide production. In conjunction, hydrogen gas opened adenosine triphosphate-sensitive potassium channel channels, which activate downstream mitogen-activated protein kinase pathways. Growing molecular mechanisms have discovered a plethora of downstream targets for hydrogen gas therapy that include autophagy (*via* the adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin pathway), histone modification, mitochondrial unfolded protein response, acute oxidative stress after exercise, and oxidative stress secondary to aging. In conclusion, evolving research has discovered novel molecular connections that will continue to widen applications for hydrogen therapy.

Key words: hydrogen therapy; selective anti-oxidation, therapeutic applications; hydrogen pathway; anti-inflammation; anti-apoptosis; hydrogen gas; future directions

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Introduction

Clinical and pre-clinical studies have reported a broad range of applications for hydrogen gas therapy. Molecular hydrogen is the smallest gas molecule that can cross cellular membranes and diffuse throughout the body; however, it requires proper safety measures at concentrations above 4% because of its combustible properties.[1] The reaction constants of hydrogen with oxide radical ion ($\bullet O^-$) and hydroxyl radical ($\bullet OH$) in water is in the order of 10^{-7} M/s, compared to other molecules that are in the orders of 10^{-9} to 10^{-10} M/s; however, with high collision rates, hydrogen may overcome the low reaction rate constants and be a therapeutic antioxidant.[1,2] Classically, conventional antioxidant therapy was limited because it neutralized both the detrimental and protective effects of reactive oxygen species (ROS): strongly oxidizing ROS, *e.g.*, $\bullet OH$, which damage tissue and advantageous species, superoxide and hydrogen peroxide, which enhance endogenous protective signal transduction pathways.[3] As a weak reducing agent, hydrogen gas avoids this paradox by reacting with strong oxidants, *e.g.*, $\bullet OH$, while leaving other beneficial oxidants reactive.[3]

Hydrogen's unique reducing properties have been leveraged to treat a plethora of oxidative stress pathologies. Acute oxidative stress develops from vigorous exercise, inflammation, ischemia and reperfusion (I/R) injury, surgical bleeding, and tissue transplantation.[4,5] The first report of the therapeutic effects of hydrogen presented the regression of skin tumors in a mice model with squamous cell carcinoma.[6] Studies have since broadened to include cardiovascular,[7] nervous system,[8] reproductive, metabolic,[9] digestive,[10] and respiratory diseases[11] (**Figure 1**).

While 12 L of endogenous hydrogen gas is produced daily by the intestinal flora, the mechanisms of low concentration hydrogen gas (1–4%) require further investigation,[3] and although the mechanisms for hydrogen remain unclear, non-mechanistic studies have primarily focused on ischemic models. Molecular hydrogen behaves as an inert gas at body temperature and does not react with any biological compounds; therefore, with few side-effects and numerous publications on its biological and medical benefits, there is a promising potential for clinical use.[12] In this review, we hope to elucidate some of the emerging mechanisms and novel applications of hydrogen gas therapy.

Under aerobic conditions normal cellular functions produce ROS—*e.g.*, superoxide radical, $\bullet OH$ and hydrogen peroxide.[13] During ischemia, mitochondrial damage occurs, causing electrons to leak from the Krebs cycle and the electron transport chain; these electrons produce superoxide anion

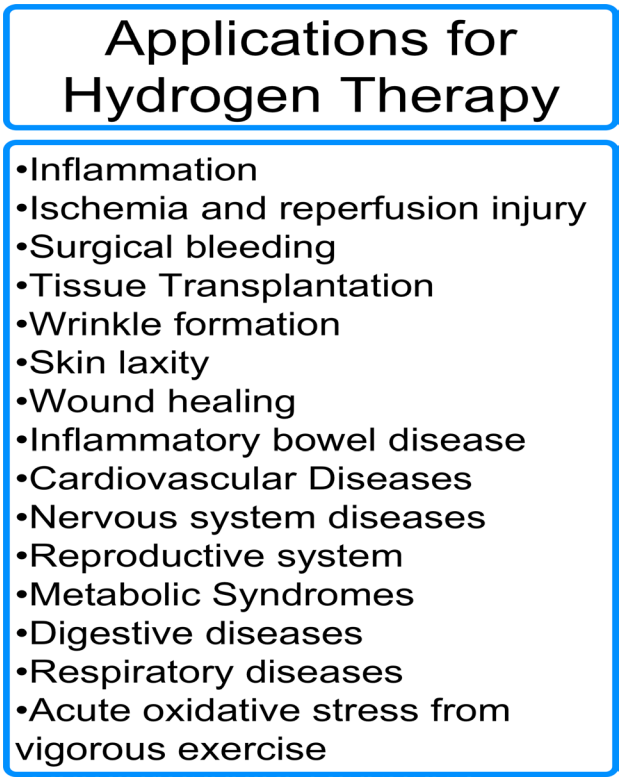

Figure 1: Table of literature supported applications of hydrogen gas therapy.

radicals (O_2^-•) which cause oxidative stress.[14] Additionally, O_2^-• is produced by metabolic oxidases, NADPH and xanthine oxidases,[13] converted into hydrogen peroxide (H_2O_2) by superoxide dismutase,[15] and then detoxified into H_2O by either glutathione peroxidase or catalase.[16] By way of the classically described Fenton Reaction, transitional ions, Fe^{3+} and Cu^{2+}, are reduced by copious amounts of O_2^-• and subsequently react with H_2O_2 to produce •OH.[17] Of importance, the strong oxidative and rapid diffusion properties of •OH lead to unpredictable non-specific reactions; in situ reactions produce oxidized bases, abasic sites, DNA-DNA intrastrand adducts, DNA strand breaks and DNA-protein cross-links.[18] Finally, the acceptance of hydrogen gas as a therapy for oxidative stress is at least in part due to its preferential reduction of stronger oxidants, *e.g.*, •OH and $ONOO^-$.[2]

Routes of Hydrogen Administration

Molecular hydrogen is usually administered by inhaling hydrogen-containing air (HCA) or by oral intake of hydrogen water (HW).[19] Inhaled hydrogen gas is most suitable for defense against acute oxidative stress in emergency situations due to its rapid mechanism of action.[16] In comparison, HCA showed superior distribution of hydrogen to both atrial and arterial blood, while HW increased hydrogen concentration by 10x in the atrial blood compared to that of the arterial blood.[19] Additional studies found support for the hydrogen mediated reduction of •OH produced *via* the radiolysis or photolysis of water.[20] Similarly, Ohsawa et al.[16] reported that hydrogen treatment (1–4% HCA) attenuated the deleterious damage of ROS, increasing neuronal survival and vitality, in both *in vitro* and *in vivo* ischemic models. Translationally, Ono et al.[21] investigated the therapeutic effects of hydrogen gas (1–4%) in a randomized clinical study in patients with acute cerebral infarction and moderate-severity National Institute of Health Stroke Scale (NIHSS) scores 2–6. In this study, magnetic resonance imaging (MRI) findings suggested a reduced brain infarction accompanied with a quicker recovery time in the hydrogen group evaluated by NIHSS scores. No adverse effects were observed.[21]

Oxidative stress induced mitochondrial damage is one of the causes of Parkinson's disease (PD).[22] Therapeutically, HW (0.08 ppm), made by dissolving electrolyzed hydrogen into pure water (H_2 bubbled water) or utilizing electrochemical reaction of magnesium with water (H_2/Mg water), a half-life of 2 hours, used for water consumption in animal models, significantly reduced dopaminergic neurons in substantia nigra after the injection with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the PD mouse model.[23] Additionally, more profound attenuation of oxidative stress and inflammatory responses was seen after treatment with HW in an Alzheimer's disease model in females compared to males[24]; however, the sexual dimorphic differences require further investigation. Propitiously, HW can be administered frequently to overcome a short half-life and shows translational promise in preventative care and treatment for ROS associated pathologies.

In the neonatal hypoxia-ischemic rat model, hydrogen saline (HS) (5 mL/kg) reduced infarction ratio, increased neuronal survival, and reduced the number of apoptotic cells in part through suppression of caspase-3 activity.[25] Paralleling these findings, early brain injury in an endovascularpuncture model of subarachnoid hemorrhage (SAH) was reduced by lowering neuronal apoptosis and associated protein markers (interleukin (IL)-6, Caspase-1, 3, IL-1β, and tumor necrosis factor-α (TNF-α)) after HS treatment (5 mL/kg).[26] In a rat model of neuropathic pain induced by L5 spinal nerve ligation, intrathecal injection of HS (20 µL of 0.6 mM HS) relieved allodynia and thermal hyperalgesia *via* reduction of spinal astrocytes and microglia, which are induced by an over abundance of •OH and $ONOO^-$.[27] Lastly, HS by intravitreal (20 µL of 400 mL saline solution with hydrogen greater than 0.6 mM) and intraperitoneal (5 mL/kg) routes reduced retinal excitotoxic injury and promoted retinal recovery by the inhibition of glial cells, decreased expression of inducible NO synthase (iNOS) and 78 kDa glucose-regulated protein (GRP78), and increased clearance of glutamate.[28]

Due to the hydrogen's ability to diffuse through the skin and into the blood stream, hydrogen bath research has grown and evolved in therapeutic applications. HW treatment can reverse the skin aging effects of ultraviolet radiation and ROS (wrinkle formation, laxity, leathery appearance, and impaired wound healing) by promoting type-1 collagen synthesis and significantly reducing human embryo fibroblast cell death after exposure to ultraviolet (UV_A).[29] Expanding the application for hydrogen gas therapy, excised cardiac grafts were submersed in a hydrogen-rich water bath (hydrogen concentration 0.5 mM) during cold preservation which attenuated cold ischemia/reperfusion injury in grafts from syngeneic older donors and allografts exposed to extended cold storage.[30] Moreover, retinal ischemia/reperfusion in-

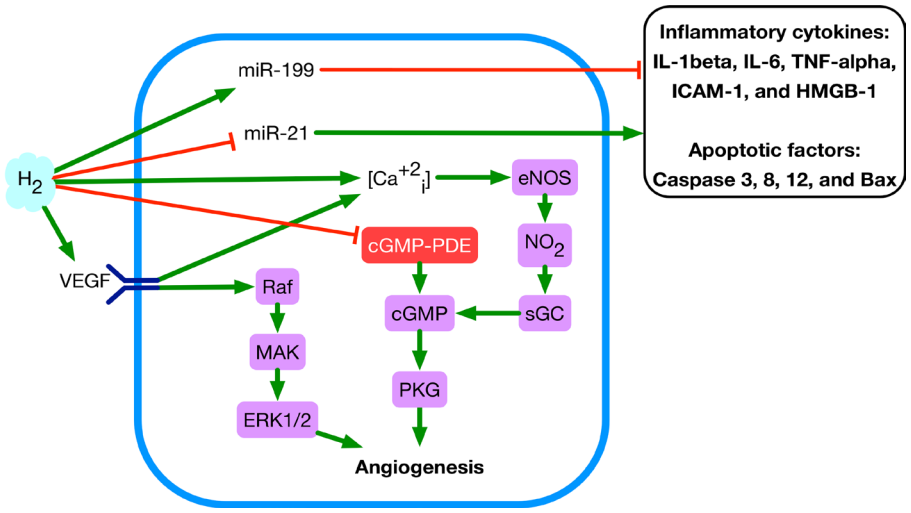

Figure 2: Proposed pathway for hydrogen (H_2) therapy and its downstream targets.
Note: VEGF: Vascular endothelial growth factor; MAK: serine/threonine-protein kinase; ERK1/2: extracellular signal-regulated kinase 1/2; cGMP: cyclic guanosine monophosphate; PDE: phosphodiesterases; PKG: protein kinase G; eNOS: endothelial nitric oxide synthase; NO_2: nitrogen dioxide; sGC: solubleguanylatecyclase; IL: interleukin; TNF: tumor necrosis factor; ICAM-1: intercellular cell adhesion molecule-1; HMGB-1: high-mobility group box protein 1.

jury in animal models by transient elevation of intraocular pressure and a mechanistic increase in ROS and subsequent apoptosis was reversed by the continuous delivery of hydrogen loaded eye drops to the ocular surface (4 mL/min, at saturated levels of hydrogen in solution) -- a significant reduction in retinal cell apoptosis.[31] Clinically, hydrogen infused eye-drops used to target retinal ischemia may also treat ischemic optic neuropathies, obstructive arterial and venous retinopathies, carotid occlusive disorders, retinopathy of prematurity, chronic diabetic retinopathy and glaucoma.[32] Current research of the direct incorporation of hydrogen has made significant therapeutic breakthroughs and, therefore, warrants further research to improve mechanistic understanding.

In inflammatory bowel disease, oxidation and inflammation activate macrophages and neutrophils which aggregate in the inflamed gut and elevate levels of ROS.[33] Mice with dextran sodium sulfate-induced colitis were given lactulose, fermented by bacteria in the colon, to produce high amounts of hydrogen, which significantly reduced colonic inflammation thus protecting the colonic mucosa.[33] Additionally, alpha-glucosidase inhibitor (acarbose) reduced postprandial hyperglycemia through the attenuation of disaccharide digestion in the small intestine and increased hydrogen fermentation of undigested carbohydrates by the resident enterobacterial flora, which was associated with a risk reduction of 25% for developing diabetes, 34% for developing *de novo* hypertension, and 49% for experiencing adverse cardiovascular events.[34,35] The currently established endogenous potential of hydrogen gas, created for the neutralization of oxidative stress associated with inflammatory and cardiovascular pathologies, provides a foundational premise for future clinical trials.

Biological Effects and Potential Mechanisms (Signaling Pathways)

As previously mentioned, hydrogen gas penetrates biomembranes, even reaching the cell nuclei, and provides selective anti-oxidant, anti-inflammatory, and anti-apoptotic properties. In hypoxic environments, HT-22 cells upregulate the expression of miR-200 family which is reversed by the presence of hydrogen.[36] Further, retinal microglia cells activated with lipopolysaccharide (LPS) and then treated with hydrogen-saturated medium showed significant downregulation of miR-9 and miR-21, while miR-199 upregulation reduced inflammatory injury.[37] Additionally, hydrogen gas downregulates the expression of pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-a, intercellular cell adhesion molecule-1 (ICAM-1), and high-mobility group box protein 1 (HMGB-1), and pro-apoptotic factors, such as caspase-3, caspase-12, caspase-8, and Bax.[5]

Furthermore, hydrogen gas has been shown to play an important role in the induction of angiogenesis which is necessary in neurogenesis, synaptogenesis, and neurosynaptic plasticity.[38] In a chicken chorioallantoic membrane, a simple model for investigating angiogenesis, hydrogen sulfide (H_2S) increased the length and complexity of the vascular network.[39] This mechanism was then studied in detail with endothelial cells *in vitro* to develop a working model for H_2S, and the following was established downstream: inhibition of cGMP phosphodiesterase led to higher levels of cGMP that activated protein kinase and promoted angiogenesis; next, as H_2S increased the levels of intracellular calcium, stimulated VEGF increased NO production.[40] In conjunction, H_2S opened KATP channels through direct interaction of Cys6 and Cys26, which activated downstream MAPK pathways.[41] A working Hydrogen pathway model can be seen in **Figure 2**. Although enlightening, these mechanistic connections also expose insufficiencies that merit additional explorative research to generate additional therapeutic targets.

Future Directions

Addressing these caveats, hydrogen gas is an attractive therapy with a growing molecular mechanism justifying the treatment of additional pathologies. With developing potential, hydrogen therapy may play a significant role in the formation of pial collaterals that sprout through angiogenesis mechanisms and require paracrine VEGF-stimulation of FIk1-Notch signaling.[42] These increased collaterals in the microcirculation are a primary clinical determinant of ischemic tissue damage after vascular obstruction.[42,43] Lastly, recent studies have discovered a plethora of downstream targets of hydrogen gas therapy that include autophagy (*via* the adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) pathway),[44,45] histone modification, mitochondrial

unfolded protein response,[46] acute oxidative stress after exercise,[47] and oxidative stress secondary to aging[48] (**Figure 3**). In conclusion, hydrogen gas has an expanding potential that is built upon an already established list of pathologies. Evolving research has discovered novel molecular connections that will continue to widen applications for hydrogen therapy. Our review suggests that a dearth of mechanistic knowledge and an already promising list of applications merit further mechanistic investigation and additional therapeutic trials for hydrogen gas.

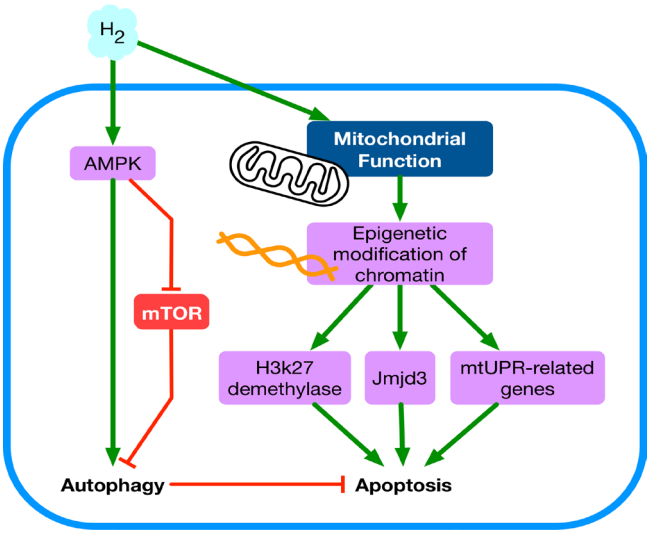

Figure 3: New targets discovered for hydrogen (H_2) therapy.
Note: The effects of hydrogen therapy are mediated in part through regulation of autophagy pathways and mitochondrial function. mtUPR: Unfolded protein response; AMPK: adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin; mTOR:mammalian target of rapamycin.

Author contributions
The idea and literature search were conducted by NM. Manuscript was drafted by NM. Critical revisions of the manuscript were made by all authors.
Conflicts of interest
The authors have no conflict of interest.
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