

Recent advances in hydrogen research as a therapeutic medical gas

CHIEN-SHENG HUANG^{1,2}, TOMOHIRO KAWAMURA^{2,3}, YOSHIYA TOYODA² & AT SUNORI NAKAO^{2,3}

¹Division of Thoracic Surgery, Department of Surgery, Taipei-Veterans General Hospital and National Yang-Ming University School of Medicine, Taipei, Taiwan, ²Heart, Lung and Esophageal Surgery Institute, and ³Thomas E Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

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Abstract

Recent basic and clinical research has revealed that hydrogen is an important physiological regulatory factor with antioxidant, anti-inflammatory and anti-apoptotic protective effects on cells and organs. Therapeutic hydrogen has been applied by different delivery methods including straightforward inhalation, drinking hydrogen dissolved in water and injection with hydrogen-saturated saline. This review summarizes currently available data regarding the protective role of hydrogen, provides an outline of recent advances in research on the use of hydrogen as a therapeutic medical gas in diverse models of disease and discusses the feasibility of hydrogen as a therapeutic strategy. It is not an overstatement to say that hydrogen's impact on therapeutic and preventive medicine could be enormous in the future.

Keywords: *Molecular hydrogen, radical oxygen species, antioxidant therapy*

Introduction

Hydrogen is the lightest and most abundant chemical element and provides the source of energy for the sun by nuclear fusion to produce helium. Since Ohsawa et al. [1] discovered that hydrogen gas has antioxidant and anti-apoptotic properties that protect the brain against ischemia-reperfusion (I/R) injury and stroke by selectively neutralizing hydroxyl radicals, hydrogen gas has come to the forefront of therapeutic medical gas research. Accumulated evidence in a variety of biomedical fields using clinical and experimental models for many diseases proves that hydrogen, administered either through gas inhalation or consumption of an aqueous hydrogen-containing solution, can act as a scavenger to selectively alleviate reactive oxygen species (ROS) and exert potent cellular protective effects (Figure 1). This review will focus on the physiological roles of hydrogen in humans, its feasibility as a therapeutic strategy and the possible mechanisms involved in its protective

effects. Additionally, the findings of recent studies of hydrogen in different disease models are summarized.

Chemistry of hydrogen and its industrial use

Hydrogen is a colourless, odourless, non-metallic, tasteless and highly combustible diatomic gas with the molecular formula H₂. Robert Boyle first produced hydrogen gas artificially by dissolving iron in diluted hydrochloric acid in 1671. Henry Cavendish recognized that hydrogen was a discrete gas and referred to it as 'inflammatory air'. Lavoisier named this property 'hydrogen' in 1783 from the Greek words *hydro*, 'water', and *genes*, 'forming'. Free hydrogen is comparatively rare on earth, as Earth's atmosphere contains less than 1 part per million of hydrogen, although hydrogen constitutes nearly 75% of the universe's elemental mass. The majority of hydrogen atoms are found in water and organic compounds. Hydrogen is highly reactive to oxygen

Correspondence: Atsunori Nakao, MD, E1551, Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA, 15213, USA. Tel: 1-412-648-9547. Fax: 1-412-624-6666. Email: anakao@imap.pitt.edu

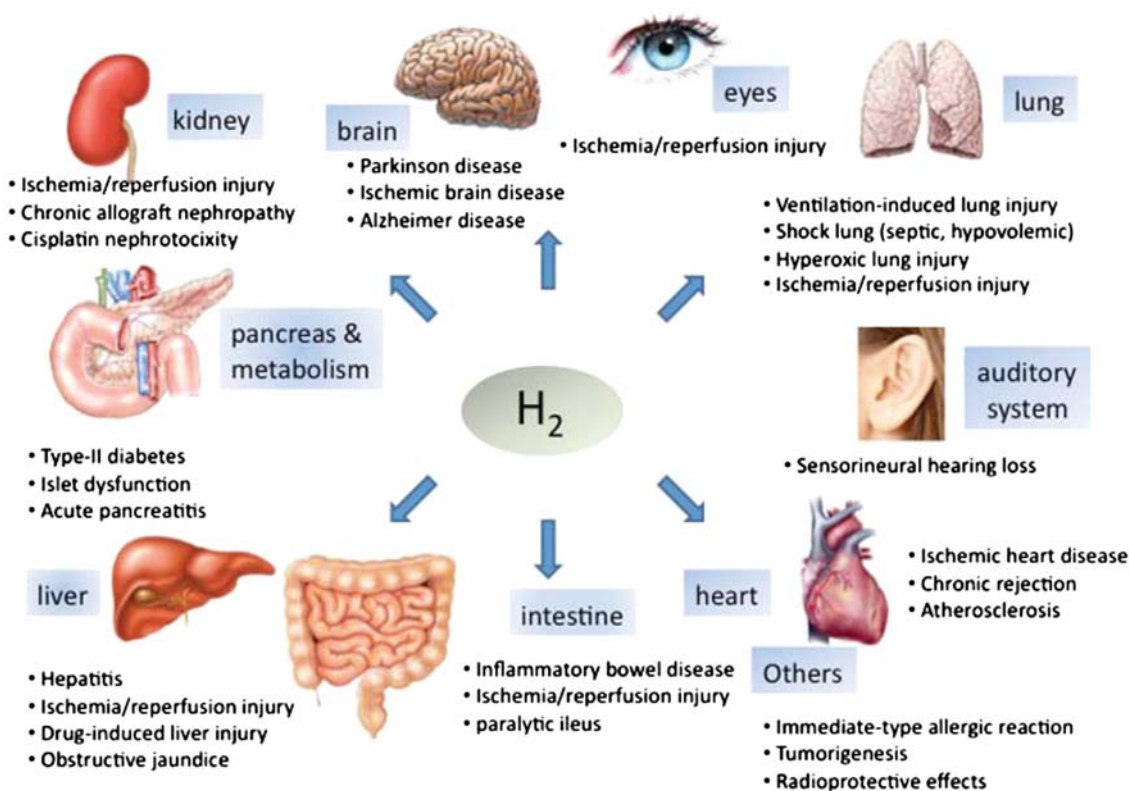


Figure 1. Therapeutic opportunities of hydrogen in a variety of disease models.

and other oxidants in the presence of specific catalysts or heat. One of the more spectacular demonstrations of its reactivity was shown in 1937 by the Zeppelin Hindenburg disaster. However, safe hydrogen concentrations in air and in pure oxygen gas are <4.6 and 4.1% by volume, respectively.

Currently, the two largest uses of hydrogen are fossil fuel processing and ammonia production for fertilizer. As one of the most highly produced inorganic chemicals, ammonia is synthesized by hydrogen and nitrogen ($3H_2 + N_2 \rightarrow 2NH_3$) and serves as a precursor to fertilizer, which is necessary to meet the nutritional needs of terrestrial organisms. Hydrogen has also been utilized as an energy carrier. Chemical energy is converted to mechanical energy either by burning hydrogen in an internal combustion engine or by reacting hydrogen with oxygen in a fuel cell to run an electric motor. Hydrogen is 3-times more energy dense by mass than gasoline (143 MJ/kg vs 46.9 MJ/kg).

Physiological roles of hydrogen molecules in humans

Hydrogen is not endogenously produced in human cells, since enzymes with hydrogenase activity do not exist in humans. However, anaerobic organisms in the large intestine obtain their energy primarily by breaking down carbohydrates, mainly from the undigested

polysaccharide fraction of plant cells and starches, via hydrogenase and generate hydrogen. More than 12 litres of hydrogen per day are continuously produced in the human body under normal physiological conditions, primarily by the fermentation of non-digestible carbohydrates by microbiota in the large intestine [2]. Once in circulation, hydrogen is only excreted via the lungs [3]. Therefore, exhalation of hydrogen forms the basis for a routinely used breath test for gastrointestinal transit and assessment of small intestinal bacterial overgrowth [4,5]. Antibiotic treatment can alter the results of H_2 -breath tests by diminishing anaerobic H_2 -producing micro-organisms [6].

Hydrogen in the human body is eliminated through three pathways: flatus, respiratory excretion after absorption into the systemic circulation and metabolism by colonic microbiota [7]. Colonic bacteria can eliminate hydrogen through three metabolic methods. They can reduce hydrogen to hydrogen sulphide ($4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$), convert hydrogen to methane ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) or use hydrogen to generate acetate ($4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$) [8,9]. Of interest, a recent study revealed that hydrogen in the human body may serve as a modulator of signal transduction, like other gaseous signalling molecules (e.g. nitric oxide (NO), CO and H_2S) [10] and hydrogen has been proposed as 'the fourth signalling gaseous molecule' [11].

Possible mechanisms of hydrogen as a therapeutic agent

Although disparate mechanisms for the tissue and cellular protection afforded by hydrogen exposure have been proposed, the role of hydrogen as an antioxidant has been advocated. The antioxidant capabilities of hydrogen include activities as a scavenger of free radicals. Hydrogen selectively reduces hydroxyl radicals ($\bullet\text{OH}$) and peroxynitrite (ONOO^-), which are very strong oxidants that react indiscriminately with nucleic acids, lipids and proteins resulting in DNA fragmentation, lipid peroxidation and protein inactivation. Biochemical experiments, using fluorescent probes and electron resonance spectroscopy spin traps, suggest that the effects of hydrogen against peroxynitrite are less potent than those against hydroxyl radicals [1]. Another possible mechanism underlying the cellular protection afforded by hydrogen may be an increase in antioxidant enzymes such as catalase, superoxide dismutase or heme oxygenase-1 [12,13]. Anti-apoptotic properties of hydrogen via inhibition of caspase-3 activation have also been postulated [14]. Hydrogen exhibited anti-inflammatory activity in various injury models. Typically, oxidative stress-induced inflammatory tissue injury is inhibited by hydrogen with down-regulation of pro-inflammatory cytokines, such as interleukin (IL)-1 β , IL-6, chemokine (CC motif) ligand 2 and tumour necrosis factor- α (TNF- α) [15,16].

Potential advantages of hydrogen therapy

Hydrogen may have a huge potential as a safe and potent therapeutic medical gas, as well as several potential advantages over current pharmacological therapies. First, hydrogen is highly diffusible and could potentially reach subcellular compartments, such as mitochondria and nuclei, which are the primary site of ROS generation and DNA damage [1], but are also notoriously difficult to target pharmacologically. Secondly, hydrogen selectively reduces detrimental hydroxyl radicals and peroxynitrite, but does not decrease the steady-state levels of nitric oxide (NO) [1]. Endogenous NO signalling pathways modulate pulmonary vascular tone and leukocyte/endothelial interactions and, therefore, it may be beneficial to spare endogenous NO [17]. This lack of reactivity to NO may enable administration of hydrogen gas with NO, which has proven clinical effectiveness. The hyporeactivity of hydrogen with other gases at therapeutic concentrations may allow hydrogen to be administered with other therapeutic gases, including inhaled anaesthesia agents [18]. Finally, hydrogen treatment did not eliminate O_2^- or H_2O_2 when tested *in vitro* [1]. Macrophages and neutrophils must generate ROS in order to kill some types of

bacteria engulfed by phagocytosis. Thus, O_2^- and H_2O_2 have important functions in neutrophils and macrophages [1]. Hydrogen therapy may spare the innate immune system and allow phagocytosis of infecting organisms. It is not clear whether a similar reaction preferentially occurs under complex biological conditions. In fact, experimental studies have demonstrated that hydrogen has potent therapeutic efficacies on both parasite infection [19] and polymicrobial sepsis [13].

Delivery of hydrogen

Inhalation

Hydrogen can be easily delivered via inhalation by delivering the gas through a ventilator circuit, face-mask or nasal cannula. This is a straightforward therapeutic option. Hydrogen poses no risk of explosion in air and in pure oxygen when present at concentrations <4%. However, safety is still a concern and the desired concentration of hydrogen must be monitored and maintained with proven, commercially available tools [20]. The safety of hydrogen for humans is demonstrated by its application in Hydrex, an exotic, breathing gas mixture of 49% hydrogen, 50% helium and 1% oxygen, which is used for prevention of decompression sickness and nitrogen narcosis during very deep technical diving [21].

Oral intake of hydrogen-rich water

Although inhaled hydrogen gas may act more rapidly, this method of administration is not practical in daily life or suitable for continuous consumption for preventive or therapeutic use. In contrast, solubilized hydrogen may be beneficial since it is a portable, easily administered and safe means of delivering molecular hydrogen [22]. Drinking hydrogen-rich water (HW) has a comparable effects to hydrogen inhalation [23]. Hydrogen water can be made by several methods, included dissolving electrolysed hydrogen into pure water, dissolving hydrogen into water under high pressure and by reaction of magnesium with water ($\text{Mg} + 2\text{H}_2\text{O} \rightarrow \text{Mg}(\text{OH})_2 + \text{H}_2$).

Injectable hydrogen-rich fluid

Even though oral administration is safe and convenient, hydrogen in water tends to evaporate over time and some hydrogen is lost in the stomach or intestine, making it difficult to control the concentration of hydrogen administered. Administration of hydrogen via an injectable hydrogen-rich vehicle may allow delivery of more accurate concentrations of hydrogen [24].

Hydrogen research in various disease models

Central nervous system

Since Ohsawa et al. [1] reported that inhaled hydrogen gas could reduce infarct size in rats of a focal cerebral I/R injury model by scavenging detrimental ROS, the efficacies of hydrogen on brain pathologies have been studied extensively. Because hydrogen can easily penetrate the blood–brain barrier by gaseous diffusion, it may be a very promising agent to protect intracranial neurons [1,21,25].

Hypoxia-ischemia (HI) insult occurs in the perinatal period and represents a major cause of brain damage in newborns. Neuronal cell death due to either necrosis or apoptosis is involved in HI-induced cerebral injury [26]. Cai et al. [24,27] reported that 2% hydrogen inhalation, as well as injection of hydrogen-saturated saline, provided brain protection via inhibition of neuronal apoptosis and reduced caspase-3 and caspase-12 activities. Additionally, these short-term effects were translated into long-term neurological and neurobehavioural functional improvements when tested 5 weeks after cerebral HI insult [24]. Conflicting results were reported by Matchett et al. [28] using models of moderate and severe neonatal brain hypoxia. Inhalation of 2.9% hydrogen did not decrease infarction volume or brain lipid peroxidation, but a trend was seen suggesting a beneficial effect on middle cerebral artery occlusion in an adult rat focal ischemia model. The results reported by Matchett et al. [28] were different from previous studies, which reported beneficial effects of hydrogen gas therapy in adult rat focal ischemia and mild neonatal HI models [1,24,27]. These divergent findings might be due to differing experimental conditions, such as different degrees of HI insult, age of pups, concentration of hydrogen and length of hydrogen exposure.

Oral intake of hydrogen-supplemented water containing a therapeutic dose of hydrogen is an alternative mode of delivery of hydrogen. Nagata et al. [25] demonstrated that consumption of hydrogen water prevents the stress-induced decline in learning and memory caused by chronic physical restraint in a mouse model [26]. Vitamin C, a non-enzymatic antioxidant, is particularly important and plenty in the brain [29]. Sato et al. [30] reported that administering hydrogen-rich water to mice that cannot synthesize vitamin C markedly decreased superoxide formation in brain slices in a hypoxia-reoxygenation model by the reducing of both hydroxyl and superoxide levels under specific conditions, such as ischemia and reperfusion *in vivo*.

The efficacies of hydrogen have also been shown in neurological degenerative disease. Fujita et al. [31] gave hydrogen-supplemented drinking water to the mice with Parkinson's disease induced by oral administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Drinking hydrogen-containing

water, even with hydrogen concentrations as low as 0.04 mM, significantly reduced the loss of dopaminergic neurons and decreased accumulation of DNA damage and lipid peroxidation, suggesting that drinking H₂-containing water may be useful in daily life to prevent or minimize the risk of lifestyle-related oxidative stress and neurodegeneration in the nigrostriatal dopaminergic pathway. Similarly, Fu et al. [32] reported the efficacies of oral intake of hydrogen water in a rat Parkinson's disease model induced by intrastriatal injection of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA). They demonstrated that administration of hydrogen, either before or after the stereotactic injection of 6-OHDA, efficiently prevented both the development and progression of nigrostriatal degeneration in rats. Thus, hydrogen may be able to retard the development and progression of Parkinson's disease.

Alzheimer's disease, another neurodegenerative disease, is the most common cause of progressive dementia in the elderly population and is associated with loss of cholinergic function through amyloid cascades, oxidative stress and hypoxia [33]. The accumulation of β -amyloid in the brain, in particular accumulation of amyloid β -42, initiates a cascade of events that ultimately leads to neuronal dysfunction, neurodegeneration and dementia [34]. Li et al. [35] reported that hydrogen-rich saline injection, intraperitoneally, daily for 2 weeks, improved the cognitive and memory functions in an amyloid β 1-42-induced Alzheimer's-like rat model, by preventing neuroinflammation and oxidative stress.

ROS play a critical role in the secondary phase of spinal cord injury, which may lead to further chronic neurodegeneration. Chen et al. [36] demonstrated that hydrogen-rich saline injection decreased oxidative stress, inflammation and apoptotic cell death and improved locomotor function after spinal cord injury in a rat model of acute spinal cord contusion injury.

Cardiovascular system

In the cardiovascular system, there is evidence that hydrogen treatment provides protection against myocardial I/R injury, cold I/R injury following heart transplantation and the development of atherosclerosis. Hayashida et al. [37] reported that during I/R injury induced by transient occlusion of the left anterior descending coronary artery in rats, inhalation of H₂ gas at levels ranging from 0.5–2% limited the extent of myocardial infarction without altering haemodynamic parameters. Another study using same model showed that injection of hydrogen-rich saline provided cardioprotection against I/R injury by decreasing plasma and myocardial malondialdehyde (MDA) concentrations, cardiac cell apoptosis and caspase-3 activity and reduced infarct size [14].

I/R injury following heart transplantation is now recognized as a major determinant of primary graft dysfunction and chronic rejection [38] and can promote the subsequent development of graft coronary artery disease [39]. Nakao et al. [18] utilized a syngeneic rat heterotopic heart transplantation model with 6 h or 18 h of cold ischemia and found that inhalation of hydrogen (2%) attenuated myocardial injury. Hydrogen treatment significantly reduced lipid peroxidation and serum HMGB-1 protein levels as compared with air-treated controls. Furthermore, dual therapy with hydrogen and CO provided cumulative protection and significantly attenuated I/R graft injury, reducing the infarcted area and decreasing in serum troponin I and creatine phosphokinase.

Atherosclerosis and related cardiovascular diseases represent a state of inflammation and oxidative stress, characterized by the accumulation of inflammatory cells and oxidized products in affected blood vessels [40]. Ohsawa et al. [41] employed an apolipoprotein E knockout mouse model, which develops atherosclerosis in a short time due to impaired clearing of plasma lipoprotein [42]. Oral ingestion of hydrogen-supplemented water (0.6 mM) *ad libitum* for 6 months prevented the development of atherosclerosis in apolipoprotein E knockout mice, in part, through its ability to limit the amount and deleterious effects of oxidative stress in the blood vessels of these mice.

Lung

Imbalances between ROS and the antioxidant defense system are involved in certain pulmonary pathologic conditions, such as lung inflammation [43,44], ventilator-induced lung injury (VILI) and acute respiratory distress syndrome [45,46]. Inhaled therapeutic gas is a reasonable approach for treatment of lung injury as it would be an easily delivered and straightforward therapeutic option. Recently, our group has shown that inhalation of 2% hydrogen gas attenuates VILI in a mouse model (presented at the American Thoracic Society 2010 International Conference, Huang et al., Hydrogen Gas Inhalation Attenuates Ventilator-Induced Lung Injury in Mice, New Orleans, 17 May 2010). Ventilation with 2% hydrogen in balanced air significantly ameliorated VILI-induced lung injury including interstitial oedema, alveolar septum thickening and infiltration of inflammatory cells (Figure 2). This data strongly suggests that inhaled hydrogen gas could therapeutically mitigate VILI via its antioxidant and anti-inflammatory effects.

Exposure to high concentrations of oxygen for prolonged periods causes hyperoxic lung injury that leads to respiratory failure. High concentrations of oxygen markedly impair lung function and increase inflammatory responses. In our studies, hyperoxia (caused by exposure to 98% O₂, 2% N₂) for 60 h caused lung oedema and led to deteriorated gas

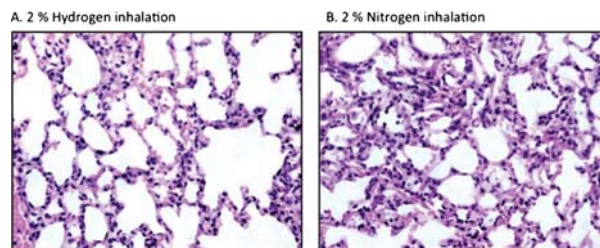


Figure 2. Haematoxylin and eosin staining of the lung histopathology from mice (400× magnification) exposed to 2 h of high tidal volume ventilation (30 ml/kg) to induce ventilator-induced lung injury (VILI). When 2% hydrogen inhalation was applied in VILI (A), less interstitial oedema, less alveolar haemorrhage, less alveolar septum thickening and fewer infiltrating inflammatory cells are observed as compared with a 2% nitrogen control (B).

exchange. Hyperoxia in the presence of 2% hydrogen (98% O₂, 2% H₂) significantly reduced hyperoxic lung injury. Interestingly, hydrogen increased the levels of heme oxygenase (HO)-1 mRNA and HO-1 protein in injured lung tissue, suggesting that the protective effects afforded by hydrogen may be mediated by HO-1 induction (Figure 3).

Acute lung injury secondary to remote organ damage and followed by deleterious systemic inflammation is a critical event. Analysing lung injury induced by intestinal I/R injury in a rat model, Mao et al. [15] reported that hydrogen-rich saline treatment decreased neutrophil infiltration, lipid membrane peroxidation, NF-κB activation and the levels of pro-inflammatory cytokines in lung tissues compared with those in saline-treated rats.

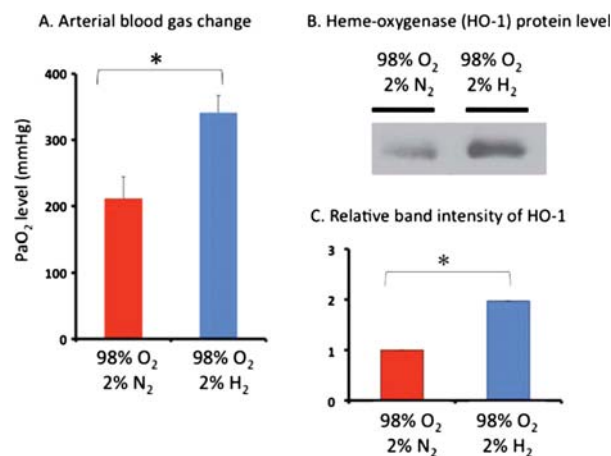


Figure 3. (A) Blood gas analysis for arterial blood after the end of 60 h of hyperoxia exposure. There was improved pulmonary function in rats exposed to hyperoxia for 60 h under 98% oxygen + 2% hydrogen as compared with 98% oxygen + 2% nitrogen controls. $n=6$ for each group, $*p < 0.05$. (B) Immunoblot analysis of heme-oxygenase (HO-1) protein obtained from rat lungs treated with hyperoxia (98% O₂ and 2% H₂ or 2% N₂) exposure for 60 h. Hyperoxia in the presence of 2% hydrogen significantly reduced hyperoxic lung injury by increasing the levels of heme oxygenase (HO)-1 protein in injured lung tissue, suggesting that the protective effects may be mediated by HO-1 induction by hydrogen.

Renal system

Cisplatin is a potent chemotherapy agent used for treatment of a broad spectrum of malignancies. The efficacy of cisplatin is dose-dependent, but the significant risk of nephrotoxicity frequently hinders the use of higher doses that may maximize its anti-neoplastic effects. At the high concentrations, cisplatin itself causes the accumulation of ROS, which may play a critical role in cisplatin-induced nephrotoxicity [47]. Naomi Nakashima-Kamimura et al. [23] reported that hydrogen-rich water alleviates cisplatin-induced nephrotoxicity, which is mediated, in part, by the accumulation of ROS that are secondary to the ability of cisplatin to inhibit the reducing form of glutathione. Despite its protective effects against cisplatin-induced nephrotoxicity, hydrogen did not impair the anti-tumour activity of cisplatin against cancer cell lines *in vitro* or tumour-bearing mice *in vivo*. Therefore, hydrogen has the potential to improve the quality-of-life of patients during chemotherapy by efficiently mitigating the nephrotoxic side-effects of cisplatin.

The vast majority of late failures in renal transplantations are attributable to chronic allograft nephropathy characterized by a progressive deterioration in renal function, increasing renal hypertension and proteinuria [48]. Cardinal et al. [22] reported that oral administration of hydrogen water prevented chronic allograft nephropathy in a rodent kidney transplantation model. Hydrogen water improved allograft function, slowed the progression of disease, reduced oxidative injury and inflammatory mediator production and improved overall survival, doing so in part by reducing oxidative stress-induced damage and reducing the activation of mitogen-activated protein kinase signalling pathways and cytokine production.

Liver

Prolonged hepatic warm ischemia aggravates oxidative stress after reperfusion and causes serious tissue damage that is characterized by elevation of hepatic enzymes and cell death. In 2007, Fukuda et al. [49] reported that inhaled hydrogen, at concentrations as low as 2–4%, reduced liver warm I/R injury in a mouse model and was associated with less oxidative stress including suppressing hepatic cell death and reducing levels of serum alanine aminotransferase and hepatic MDA.

Systemic antibiotic treatment may alter the number of protective commensal bacteria in the host's intestines, ultimately resulting in a reduced concentration of H₂ in the liver. Kajiya et al. [50] reported that H₂ released from intestinal bacteria can suppress inflammation in mouse hepatitis induced by Concanavalin A (Con A). Furthermore, they also showed that H₂ can suppress ConA-mediated T-cell activation, which

causes the tissue-destructive production of TNF- α and IFN- γ *in vitro*.

Obstruction jaundice is a life-threatening condition accompanied with endotoxemia, a systemic inflammatory response, liver injury and even multiple organ failure. Experimental and clinical studies have shown that excessive oxidative stress may promote hepatic injury in obstructive jaundice [51]. Liu et al. [52] investigated the effects of injection of hydrogen-saturated saline on liver damage due to obstructive jaundice using a rat model with ligation of the bile duct. The treatment with hydrogen-saturated saline significantly decreased serum alanine aminotransferase and aspartate aminotransferase levels, tissue MDA content, myeloperoxidase activity, pro-inflammatory cytokine levels, HMGB1 levels and histopathological changes including hepatocyte necrosis as compared with control livers from rats treated with normal saline. Additionally, hydrogen-rich saline markedly increased the activities of antioxidant enzymes (superoxide dismutase and catalase) and down-regulated extracellular signal-regulated kinase (ERK)-1/2 activation.

Pancreas

Severe acute pancreatitis is characterized by cell membrane disruption, oedema, interstitial haemorrhage, necrosis and inflammation responses and is caused by conversion of pro-enzymes to their active forms within the acinar cells. ROS and their derivatives may play important roles in triggering severe acute pancreatitis as well as in activating and recruiting neutrophils and macrophages. In a rat model, L-arginine can induce acute pancreatitis as characterized by severe pancreatic interstitial oedema, vacuolization, prominent neutrophil infiltration, acinar cell injury and necrosis in the pancreas [53]. Chen et al. [54] demonstrated that hydrogen-rich saline infusion into the tail vein significantly attenuated the severity of L-arginine-induced acute pancreatitis in rats by ameliorating increases in serum amylase activity and inhibiting neutrophil infiltration, lipid oxidation and pancreatic tissue oedema.

Intestine

Intestinal I/R injury occurs frequently in a variety of clinical settings including surgical treatment for abdominal aortic aneurysm, mesenteric artery occlusion, cardiopulmonary bypass, bowel strangulation, neonatal necrotizing enterocolitis haemorrhagic shock and small intestinal transplantation [55–57]. Buchholz et al. [58] have utilized an orthotopic, syngeneic, small intestinal transplant model in rats and shown that hydrogen treatment ameliorates transplant-induced intestinal injuries including mucosal erosion and

mucosal barrier breakdown. Perioperative inhalation of 2% hydrogen mitigated intestinal dysmotility following transplantation, reduced up-regulation of inflammatory mediators and was associated with significantly diminished lipid peroxidation as compared with air-treated grafts. Although small intestine transplantation has been recognized as a therapeutic option for the treatment of patients with intestinal failure, poor preservation and I/R injury during transplantation are still major causes of recipient morbidity and mortality. Administration of perioperative hydrogen gas may be a potent and clinically applicable therapeutic strategy for intestinal transplantation.

Dextran sodium sulphate (DSS)-induced rodent colitis is a well-established animal model of human inflammatory bowel disease, particularly ulcerative colitis [59]. DSS will interfere with the barrier function of epithelial cells and then expose the lamina propria to luminal bacterial antigens. When animals were exposed to DSS via drinking water, DSS-induced colitis was characterized by weight loss, diarrhoea or grossly bloody stool and intestinal inflammation on histopathological examination [60]. Oral intake of hydrogen water (0.78 mM) prevented the development of DSS-induced colitis in mice [61]. The administration of H₂ remarkably reduced the clinical symptoms of DSS-induced colitis and H₂ prevented DSS-mediated destruction of epithelial crypt structure, evident by histopathological evaluation.

Chen et al. [62] demonstrated that hydrogen treatment in rats, via tail vein injection of hydrogen-rich saline, had protective effects against intestinal contractile dysfunction and damage induced by intestinal warm I/R injury. These protective effects are possibly due to the ability of hydrogen to inhibit I/R-induced oxidative stress and apoptosis and to promote epithelial cell proliferation. The same group also used a similar model, administering hydrogen-rich saline via jugular vein injection, and reported that hydrogen-rich saline reduced inflammation and oxidative stress as evidenced by reducing I/R injury scores, serum diamine oxidase (DAO) activity, TNF- α , IL-1 β and IL-6 levels, tissue MDA levels and protein carbonyl and myeloperoxidase activity [63].

Recently, extensive research has been performed in the area of irritable bowel syndrome (IBS). Patients with IBS and predominantly hydrogen-producing bacteria have a higher incidence of diarrhoea than patients with IBS and predominantly methane-producing bacteria [64]. Additionally, antibiotic eradication of bacterial overgrowth in IBS with dominant hydrogen producers results in symptomatic relief from diarrhoea, suggesting that H₂ produced by colonic bacteria induced hypercontractility [65]. Thus, hydrogen-producing bacteria may be a therapeutic target for treatment of patients with IBS, especially in those with diarrhoea as a symptom.

Eyes

Transient elevation of intraocular pressure, such as acute angle-closure glaucoma and retinal artery occlusion, will lead to retinal I/R injury and can induce necrosis and apoptosis of cells leading to significant reduction in the thickness of multiple layers of the retina [66]. Oharazawa et al. [67] prepared H₂-loaded eye drops (0.8 mM, pH 7.2) by dissolving H₂ gas into saline to saturated level and then administered the H₂-loaded eye drops to the ocular surface continuously (4 L/min) during the ischemia or reperfusion periods. The dropper, connected to the bag with H₂-loaded eye drops, was held close to the rat's eye and drops were applied to the ocular surface. The hydrogen in the eye drops was found to immediately penetrate the vitreous body leading to elevated intravitreal H₂ concentration after administration. Thus, H₂-loaded eye drops could effectively protect the retina from I/R injury by scavenging hydroxyl radicals and have an enormous impact via the topical application of H₂ solution.

Auditory system

ROS can result in cellular injury in the cochlea and sensorineural hearing loss [68]. Kikkawa et al. [69] utilized antimycin A, an inhibitor of mitochondrial respiratory chain complex III, to directly damage the cochlear hair cells *in vivo* by generating ototoxicity via *in situ* ROS. Using this model, they reported that a hydrogen-saturated medium with high concentrations of directly dissolved hydrogen (1.3 \pm 0.1 mg/l), significantly alleviated ROS generation, in particular the generation of cellular hydroxyl radicals, and subsequent lipid peroxidation in the auditory epithelia and led to increased survival of the hair cells.

Allergic reactions

Itoh et al. [70] demonstrated using a mouse model that drinking hydrogen-rich water (0.8 mM) could attenuate an immediate-type allergic reaction by suppressing phosphorylation of Fc ϵ RI-associated Lyn and its downstream signalling molecules, which subsequently inhibited NADPH oxidase activity and reduced the generation of hydrogen peroxide [70]. This hints that the beneficial effects of hydrogen are not only imparted by its radical scavenging activity, but also by modulating a specific gaseous signalling pathway.

Metabolism

Metabolic syndrome is characterized by cardiometabolic risk factors that include obesity, insulin resistance, hypertension and dyslipidemia; is associated with an increased risk of developing cardiovascular

disease and type II diabetes [71,72]; and remains a serious concern in the US. Recently, oxidative stress has been implicated as playing a critical role in the pathogenesis of metabolic syndrome [73,74]. There are several studies showing protective effects of hydrogen for metabolic disorders. Kajiyama et al. [12] treated patients with type-2 diabetes with hydrogen-dissolved in water (900 ml/day) for 8 weeks. Drinking the hydrogen water resulted in reduced levels of several biomarkers of oxidative stress, including oxidized low-density lipoprotein (LDL) cholesterol in plasma and 8-isoprostanes in urine and improved glucose metabolism in patients with type 2 diabetes. A pilot study conducted in Canada demonstrated that hydrogen-rich water produced by placing metallic magnesium into drinking water was also effective in attenuating oxidative stress in human subjects with potential metabolic syndrome [75]. Drinking hydrogen-rich water (0.55–0.65 mM, 1.5–2 L/day) for an 8-week period resulted in a 39% increase in the antioxidant enzyme superoxide dismutase and a 43% decrease in thiobarbituric acid-reactive substances in urine. Further, subjects demonstrated an 8% increase in high-density lipoprotein (HDL)-cholesterol and a 13% decrease in total cholesterol/HDL-cholesterol from baseline to week 4. Thus, drinking hydrogen-rich water represents a potentially novel therapeutic and preventive strategy for metabolic syndrome.

Acarbose, an α -glucosidase inhibitor, is a pharmacological agent that specifically reduces post-prandial hyperglycaemia through retardation of disaccharide digestion, thereby reducing glucose absorption by the small intestine. Suzuki et al. [20] observed 11 healthy subjects who were treated with acarbose at a dose of 300 mg/day (100 mg three times a day) and discovered that acarbose treatment significantly increased the amount of exhaled H_2 as compared with exhaled H_2 levels before treatment with acarbose. Based on these observations, they proposed that this α -glucosidase inhibitor may unexpectedly reduce the risk of cardiovascular disease in patients with impaired glucose tolerance or type-2 diabetes and that these benefits can be attributed, at least in part, to the ability of these drugs to neutralize oxidative stress by increasing the production of H_2 in the gastrointestinal tract.

Infection

Excessive production of ROS and reduced antioxidant defense systems play an important role in the pathogenesis of infection and sepsis [76]. In 2001, Gharib et al. [19] found that breathing a hydrogen atmosphere had a striking anti-inflammatory effect in mice with schistosomiasis-associated chronic liver injury as assessed by less fibrosis, improved haemodynamics, increased nitric oxide synthase II activity, increased antioxidant enzyme activity, decreased lipid peroxide levels and decreased circulating TNF- α levels. Xie

et al. [13], in 2009, also demonstrated that hydrogen gas treatment ameliorated polymicrobial sepsis and sepsis-associated organ damage in mice through the ability of hydrogen gas to decrease the levels of oxidative products, increase the activities of antioxidant enzymes and reduce the levels of high-mobility group box 1 (HMGB1) in serum and tissue.

Tumorigenesis

A growing number of studies have found that human tumour cells can produce more ROS than non-transformed cell lines, which imparts cancers with the potential to increase cell proliferation, DNA synthesis, angiogenesis, invasion and distal metastasis [78–80]. In 1975, Dole et al. [81] noted that hyperbaric hydrogen therapy could reduce the size of skin tumours in hairless albino mice with squamous cell carcinoma. They treated the mice in a chamber with a mixture of 2.5% oxygen and 97.5% hydrogen at a pressure of 8 atmospheres for 2 weeks and observed that tumours in the mice had turned black, dropped off, shrunk and even disappeared in the hydrogen therapy group when compared with the control group. Saitoh et al. [82] reported that platinum nanocolloid-supplemented hydrogen-rich water, through its antioxidant activity, exerted a quicker antioxidant action and preferentially inhibited the clonal growth of human tongue carcinoma cells as compared with normal cells.

Radioprotective effects

Although radiation therapy is widely applied clinically as a curative or adjuvant treatment for certain types of cancer, exposure to ionizing radiation can produce severe endothelial cell injury and apoptosis in the gastrointestinal tract and haematopoietic system [83]. Qian et al. [84] reported that treating cells with hydrogen-rich cultured media before irradiation could significantly inhibit ionizing irradiation-induced human lymphocyte (AHH-1) cell apoptosis and increase cell viability *in vitro*. Intraperitoneal injection of hydrogen-rich saline before radiation in a mouse model also protected the gastrointestinal endothelia from radiation-induced injury, decreased plasma MDA and intestinal 8-8-hydroxydeoxyguanosine levels and increased plasma levels of endogenous antioxidant enzymes including superoxide dismutase and glutathione-S-transferase.

Acute radiation syndrome is caused by damage to organ tissue by excessive exposure to ionizing radiation. It is generally accepted that ionizing radiation interacts with water molecules in the body and produces a variety of active free radicals, more than half of which are hydroxyl radicals capable of ionizing radiation-induced cellular damage [85]. Because hydrogen gas has the ability to selectively scavenge hydroxyl radicals, Liu et al. [86] proposed that hydrogen gas,

especially in the form of hydrogen water, may have a promising, critical role as a novel radioprotectant in the preventive medicine.

Adverse effects of hydrogen therapy

To provide evidence of the safety of hydrogen dissolved in water (0.45–0.57 mM), Saitoh et al. [87] assessed the possible adverse effects of hydrogen dissolved in water included the mutagenicity, genotoxicity *in vivo* and subchronic oral toxicity in a rat model (20 ml/kg/day for 28 days via intragastric infusion). There were a few statistically significant changes in hematology (e.g. basophil ratio in female rats) and clinical chemistry parameters (e.g. decreased aspartate aminotransferase and decreased alanine aminotransferase in male rats). However, these changes were not considered biologically significant because the differences were negligible, occurred only in one sex and occurred within the normal clinical ranges. A significant increase in the absolute weight of the spleen was observed in the females in the hydrogen water group, but the weight ratio of the spleen to the body was not changed and no significant changes were found in either necropsy or histopathological examination.

In humans, similar clinical chemistry parameters were observed including decreases in aspartate aminotransferase and alanine aminotransferase and increases in gamma-glutamyl transferase and total bilirubin in the group receiving hydrogen-supplemented drinking water. All of these parameters remained within an acceptable clinical range. In addition, six adverse events, experienced by four people (20.0%), were assessed by the investigator as having a possible relationship to hydrogen exposure. These adverse events included loose stools (in three of 20 people), increase in frequency of bowel movement (in one person), heartburn (in one person) and headache (in one person) [75].

Future directions

The concept of using hydrogen gas for therapeutic purposes is a new field of investigation. Therefore, there is very limited information on the pathways and processes regulated *in vivo* by the hydrogen molecule. Given the previously reported data, the beneficial effects of hydrogen are undoubtedly due, in part, to its radical scavenging properties, although the direct free radical-scavenging activities of hydrogen should be more thoroughly investigated by additional studies. However, scavenging properties are unlikely to be the only explanation and undefined biological mechanisms of hydrogen as a signalling molecule may be involved. Future studies are needed to elucidate the detailed mechanisms of hydrogen as a biological

molecule. Based on the results of basic research, appropriately designed, large-scale and prospective clinical studies are warranted to optimize dose, timing and delivery methods. In addition, a more comprehensive understanding of the pharmacokinetics, biology and toxicity of hydrogen will certainly help us to harness the protective potential of hydrogen gas prior to clinical application.

Summary

Hydrogen treatment is a promising potential therapeutic option for treatment of a variety of diseases. Gas inhalation as disease therapy has received recent interest and the list of therapeutic gases continues to grow. Although further investigations are required, hydrogen may have a huge impact as a novel and innovative therapeutic tools for unmet medical needs that currently cause considerable health burdens.

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