Chapter 6
Therapeutic Effects of Hydrogen on Different Diseases

Liren Qian, Jianliang Shen and Xuejun Sun

Abstract Since 2007, hydrogen gas biology has become hotspot in medical research because of its selective antioxidant effects reported by Ohsawa et al. By the end of 2014, more than 400 papers have been published in related area, including organ ischemia-reperfusion injury, diabetes, atherosclerosis, hypertension, cancer, and other major human diseases. Many of these studies were based on the premise that the hydrogen gas is a new type of antioxidant substance, and also there are a lot of evidences of its antiapoptotic, anti-inflammatory effects. Since oxidative stress is related to almost all cells, tissues, and organs, it is related to almost all diseases. In this chapter, we focus on some typical types of diseases, including nervous system disorders, liver diseases, metabolic diseases, and some clinical studies in order to facilitate the readers to understand the molecular biology of hydrogen.

Keywords Hydrogen · Therapy · Nervous system · Metabolic · Liver · Ophthalmic · Respiratory · Inflammatory · Translational

In 2007, Ohsawa et al. found the selective antioxidative and therapeutic effects on cerebral ischemia of hydrogen gas [1]. From 2007 to now, hydrogen gas was demonstrated effective in more than 60 kinds of human diseases, most of which are oxidative stress-related diseases, including neonatal cerebral hypoxia, Parkinson's disease, and tissue ischemia-reperfusion (I/R; spinal cord, heart, lung, liver, kidney, and small intestine) [2]. Despite there are many doubts, selective antioxidative effect remains the main mechanism of hydrogen which is currently accepted. In addition, its antiapoptotic, anti-inflammatory effects play important roles in studying its biological effects.

Although there were reports of antioxidative effects of hydrogen gas as early as 1975 and 2001 [3, 4], the hydrogen gas used in the study in 2001 was with 800 kPa and 14 days, while the hydrogen gas used in the study in 2007 was with 2 kPa and

X. Sun (✉)
Department of Navy Aeromedicine, Second Military Medical University, Shanghai, China
e-mail: sunxjk@hotmail.com

L. Qian · J. Shen
Department of Hematology, Navy General Hospital, Fucheng Road, Beijing, China

X. Sun et al. (eds.), *Hydrogen Molecular Biology and Medicine,*
DOI 10.1007/978-94-017-9691-0_6

less than 1 h [1]. The two studies were definitely different with entirely different nature. The article in 2007 attracted international attention rapidly after its publication. The number of articles about hydrogen biology was with explosive growth after its publication. The paper in 2007 has been cited more than 300 times. Although most of these papers were trying to prove the therapeutic effects of hydrogen, there were two papers that reported its ineffectiveness on severe neonatal hypoxia–ischemia and disuse muscle atrophy [5, 6].

Conventional treatment options are always ineffective for severe diseases. Hydrogen is also not an exception. Although it was suggested that oxidative stress is involved in the pathogenesis of disuse muscle atrophy [7], hydrogen was ineffective on this kind of disease, which suggest that oxidative stress may not be the most important pathogenesis of disuse muscle atrophy. Therapeutic effects of hydrogen on organ I/R injury and inflammation-related diseases are the most significant.

Hydrogen is the simplest atom in nature, which consists of a proton and extranuclear electron. Hydrogen gas is the simplest molecule in nature that is composed of two hydrogen atoms. When hydrogen gas and oxygen gas are mixed, combustion or explosion may occur at concentration ranging from 4 to 75%. However, hydrogen gas is chemically inactive at room temperature and cannot react with other substances easily. Despite of this, hydrogen gas is a small molecule that can easily diffuse into body and cells, leading to high probability of collision between hydrogen and other atoms or molecules. The powerful diffusion capability of hydrogen gas perhaps makes up for the low reaction rate of hydrogen gas. This makes hydrogen gas not only stable but also selectively antioxidant, which is the special advantage of hydrogen gas.

Reactive oxygen species (ROS) play an important role in the pathophysiology of various types of cardiovascular and cerebrovascular diseases, such as stroke and myocardial infarction, metabolic diseases such as diabetes, atherosclerosis, and other important human acute and chronic diseases. It exists as two forms, oxygen-free radicals and non-oxygen-free radicals. Oxygen-free radicals include hydroxyl radical, superoxide anion, nitric oxide, nitrite anion, and other substances. Under physiological conditions, ROS are continuously generated in the body and are also continuously cleared, which is in dynamic equilibrium. However, under pathological conditions such as ischemia and inflammation, a large amount of ROS may be produced. Hydroxyl radicals and peroxynitrite are the main substances that make oxidative damage to cells. The toxicities of nitric oxide, superoxide anion, and hydrogen peroxide and other substances are weak, but they play an important role in signal transduction. In the treatment of oxidative damage previously, reductive drugs may lead to new imbalance of oxidative-redox state which is the main reason for current poor effects of antioxidant treatments. In 2007, Ohsawa et al. demonstrated that hydrogen could selectively reduce toxic hydroxyl radicals and peroxynitrite anion [1], with little effect on the ROS which have important biological functions with low toxicity. This is the selective antioxidative effect of hydrogen gas which provides a new idea for the antioxidant therapy.

As early as in 2001, Gharib et al. reported inhalation of 8 atm of H_2 has therapeutic effects on liver inflammation caused by *Schistosoma japonicum* infection [4]. They demonstrated that direct reaction between hydrogen and hydroxyl radical

is the basis of anti-inflammatory effects of hydrogen. In 2009, Kajiya et al. reported hydrogen could obviously inhibit colonic inflammation induced by dextran sulfate sodium [8]. The anti-inflammatory effect of hydrogen is related to the inhibition of the generation of ROS and proinflammatory cytokine release. In addition, macrophages play an important role in inflammation and immune regulation. Regulation on macrophages laid the foundation for its anti-inflammatory effect.

In 2008, it is found that hydrogen can reduce tissue injury in rats with hypoxia–ischemia model. Inhalation of low concentrations of hydrogen can reduce the activity of caspase-3 and caspase-12 enzyme, which suggested that it has antiapoptotic effect [9]. Because of its antiapoptotic effect, the studies of I/R injury, inflammation, and other diseases are attracting more and more attention. But the exact mechanism of antiapoptotic effect of hydrogen is not completely clear, which needs further study.

ROS play an important role in the angiogenesis process. Studies have shown that ROS can induce vascular smooth muscle hyperplasia. Kubota et al. reported that hydrogen-rich water eye drops have anticorneal angiogenesis effects [10]. The mechanism may be related to its ability of scavenging ROS generated after corneal injury, reduction of nuclear transcription factor gene transcription (NF-κB), and reduction of vascular endothelial growth factor expression levels. Angiogenesis is not only important to the pathophysiology of many vascular diseases but also play important role in the occurrence of tumor. The role of hydrogen on angiogenesis is worthy of further discussion.

6.1 Therapeutic Effects of Hydrogen on Central Nervous System Diseases

Central nervous system is one of the body's most important systems. Central nervous system disorders include acute and chronic central nervous system disorders. Acute central nervous system disorders include trauma and cerebrovascular diseases. Chronic central nervous system disorders include various types of neurodegenerative diseases. Since the biological effects of hydrogen were found, it was demonstrated has significant protective effects on central nervous system dysfunction including cerebral vascular disease, Parkinson's disease, and Alzheimer's disease [11–17]. Preliminary clinical studies have been carried out in cerebral ischemia and Parkinson's disease, which indicate that clinical application of hydrogen in neurological disease attracted great attention [13, 17].

6.1.1 Therapeutic Effects of Hydrogen on Cerebral Vascular Disease

Cerebrovascular disease is a kind of disease involving brain artery or carotid artery, leading to intracranial blood circulation disorders and brain tissue damage. Cerebrovascular disease can be divided into ischemic cerebrovascular disease and

hemorrhagic cerebrovascular disease, including cerebral infarction and cerebral embolism. No matter cerebral ischemia or cerebral hemorrhage, there are still a few effective treatments. Although the pathophysiology of cerebrovascular disease is complex, there is a similar pathophysiological process in most types of acute brain injury, such as hypoxia–ischemia, reperfusion injury, calcium overload, free radicals, and inflammatory injury, and so on. Free radicals and inflammation damage are in the heart of the pathophysiology process described above, which is the main reason why hydrogen was studied in various types of cerebrovascular disease. In 2007, Ohsawa et al. reported that inhalation of hydrogen gas exerts therapeutic effects in a rat model with left middle cerebral artery occlusion [1]. After that report, Cai et al. demonstrated that inhalation of hydrogen gas has desired therapeutic effects on hypoxic–ischemic brain damage caused by asphyxia [9].

Although some scholars reported that the therapeutic effects of hydrogen on severe brain hypoxic–ischemic injury in not ideal [5], more studies have shown that hydrogen has protective effects on brain injury caused by cardiac arrest which further confirmed the protective effects of hydrogen on hypoxic–ischemic brain injury [18].

Edaravone is currently the only approved drug for stroke [19]. Ono et al. carried out a clinical research and studied the therapeutic effects of combined hydrogen saturated saline and edaravone on brainstem infarction patients [13], compared with those patients treated with edaravone alone. It was found that magnetic resonance imaging (MRI) indicators of those patients treated with hydrogen and edaravone were better than those treated with edaravone alone. The main mechanism of edaravone is antioxidation, which is very similar to the hydrogen gas. The results suggested, hydrogen has desired therapeutic effects. This is the only clinical study currently reported treating cerebral ischemia by hydrogen.

Intracranial hemorrhage is a kind of cerebrovascular disease which very easily leads to the death, including cerebral hemorrhage and subarachnoid hemorrhage. It has been demonstrated that hydrogen has ideal protective effects on early brain injury, nerve cell necrosis, edema, and vascular spasm caused by cerebral hemorrhage and subarachnoid hemorrhage [20, 21]. This shows that hydrogen has a potential therapeutic value in all kinds of cerebral vascular diseases. Recently, hydrogen was found has therapeutic effects on platelet aggregation which needs to be paid more attention [22], because delayed hemorrhage is a serious complication of cerebral vascular disease which is lethal. But surprisingly, some researchers have proved that cerebral ischemia-induced hemorrhage could be reduced by hydrogen [23]. Apparently, these contradictory issues need further study.

6.1.2 Therapeutic Effects of Hydrogen on Neurodegenerative Diseases

Dopaminergic neuronal death at the substantia nigra pars compact may cause Parkinson's disease, which is always secondary to other neurodegenerative diseases such as Alzheimer's and other diseases. There are two mechanisms that underlie

Parkinson's disease: excessive oxidative stress and abnormal ubiquitin-proteasome system [24].

Dopamine is a kind of neurotransmitter, which is a pro-oxidant by itself, and dopaminergic cells are destined to be exposed to high concentrations of radical oxygen species. Hemi-Parkinson's disease model can be made by stereotactically injecting catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) in the right striatum [16]. The development of hemi-Parkinson's symptoms can be completely abolished by administration of hydrogen-rich water 1 week before surgery, and 40.2% of dopaminergic neurons on the toxin-injected side were reduced, whereas hydrogen treatment improved the reduction to 83.0%. Hemi-Parkinson's symptoms were also suppressed by giving hydrogen-rich water 3 days after surgery, but not as much as those observed in pretreated rats. Pretreated rats were also sacrificed 48 h after toxin injection and the tyrosine hydroxylase activity at the striatum, where dopaminergic neurons terminate. This indicated that hydrogen did not directly detoxicate 6-OHDA but exerted a delayed protective effect for dopaminergic cells. Fujita et al. also demonstrated a similar prominent effect of hydrogen-rich water on an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease [15]. MPTP is a neurotoxin that blocks complex I of the mitochondrial electron transport system and causes Parkinson's disease in mice and humans. It is interesting to note that the concentration of hydrogen that they used for the MPTP mice was only 0.08 ppm, which is the second lowest among all the trials published to date for rodents and humans. The lowest hydrogen concentration ever tested is 0.048 ppm in the dialysis solution for patients receiving hemodialysis [25].

Alzheimer's disease is the most common neurodegenerative disease which is characterized by abnormal aggregation of β-amyloid (Aβ) and tau, the large aggregates of which are respectively recognizable as senile plaques and neurofibrillary tangles [26]. Three models have been used to study therapeutic effects of hydrogen on Alzheimer's disease. A mouse model of dementia by restricting movement of mice for 10 h a day was made by Nagata et al. [27]. Cognitive functions were analyzed by passive avoidance learning, object recognition tasks, and the Morris water maze. Administration of hydrogen-rich water was demonstrated efficiently ameliorated cognitive impairment. They also showed that neural proliferation in the dentate gyrus was restored by hydrogen. Another rat model was made by intracerebroventricular injection of Aβ1-42 by Li et al. [11]. They found that intraperitoneal injection of hydrogen-rich saline for 14 days efficiently ameliorated cognitive decline and preserved long-term potentiation (LTP) by suppression of abnormal activation of IL1β, c-Jun N-terminal kinases (JNK), and NF-κB [12]. The third model was established by using a senescence-accelerated mouse prone 8 (SAMP8) strain which can exhibit early aging syndromes, including impairment in learning ability and memory [28]. The administration of hydrogen-rich water was demonstrated efficiently prevented cognitive decline by the Morris water maze.

Chorea is a kind of relatively common neurodegenerative disease. Its clinical manifestation is different from senile dementia and Parkinson's disease, but the pathophysiological processes are similar. There is still no report about the therapeutic effects of hydrogen on this disease.

6.2 Therapeutic Effects of Hydrogen on Metabolic Diseases

6.2.1 Diabetes

In 2008, Kajiyama et al. performed a randomized, double-blind, placebo-controlled study in 30 patients with diabetes mellitus type II and 6 patients with impaired glucose tolerance [29]. The patients consumed either hydrogen-rich water or placebo water for 8 weeks, with a 12-week washout period. They measured 13 biomarkers to estimate lipid and glucose metabolisms.

All the biomarkers were favorably changed with hydrogen, but statistical significance was observed only in improvement of electronegative charge-modified low-density lipoprotein (LDL) cholesterol, small dense LDL, and urinary 8-iso-prostanes. In four of six patients with impaired glucose tolerance, hydrogen normalized the oral glucose tolerance test. Lack of statistical significance in their studies was likely due to the small number of patients and the short observation period. The lack of statistical significance, however, may also suggest a less prominent effect in human diabetes mellitus compared to rodent models [30, 31].

6.2.2 Metabolic Syndrome

An open-label trial in 20 subjects with potential metabolic syndrome was performed by Nakao et al. [32]. Hydrogen-rich water was produced by placing a metallic magnesium stick in water, which yielded 0.55–0.65 mM hydrogen water (70–80% saturation). The participants consumed 1.5–2.0 L of hydrogen water per day for 8 weeks and showed a 39% increase in urinary superoxide dismutase [29]; a 43% decrease in urinary thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation; an 8% increase in high-density lipoprotein (HDL) cholesterol; a 13% decrease in total cholesterol/HDL cholesterol; and a 13% decrease in total cholesterol/HDL cholesterol. The aspartate aminotransferase [33] and alanine transaminase (ALT) levels remained unchanged, whereas the gamma-glutamyl transferase (GGT) level was increased by 24% but was still within a normal range. Although the study was not double blinded and placebo controlled, improvements in biomarkers were much more than those in other hydrogen studies in humans. As this study used a large amount of hydrogen water, the amount of hydrogen might have been a critical determinant. Alternatively, excessive hydration might have prevented the participants from excessive food intake.

6.3 Therapeutic Effects of Hydrogen on Liver Diseases

Early in 2001, there was a report about the protective effects of high-pressure hydrogen gas on liver injury [4]. In 2007, therapeutic effects of hydrogen on hepatic ischemia were further studied [1]. Therefore, the study of hydrogen gas on liver

disease is relatively early; the research methods and the types of liver diseases are various.

6.3.1 Early Studies of Hydrogen on Liver Diseases

Hydrogen was applied in the field of liver diseases prominently. Gharib et al. examined the effects of molecular hydrogen on a mouse model of schistosomiasis-associated chronic liver inflammation. The mice breathed hydrogen–oxygen mixed gas for 14 consecutive days (87.5% hydrogen gas) [28]. Liver function, liver tissue oxidative damage, fibrosis, and inflammatory indexes were observed. They demonstrated that continuous high-pressure hydrogen breath has significant protective effects on liver tissue damage, inflammation, and fibrosis. This study was groundbreaking; it is the first proof that hydrogen has anti-inflammatory, anti-hepatic fibrosis effects. However, there are still two obvious deficiencies in the study. First, the dosage is too large. Second, this study cannot replace academic status of the study which has been published articles in 2007 in *Nature Med* [1]. Because the latter proved breathing 2% hydrogen for 35 min has biological effects which is much more convenient to use. There is also no reasonable explanation in the study in 2001, which led to little attraction from other researchers.

6.3.2 Therapeutic Effects of Hydrogen on Liver Diseases

Japanese researchers published the first paper that demonstrated the therapeutic effects of low-dose hydrogen on cerebral I/R injury. Subsequently, an article reported the therapeutic effects of hydrogen on liver I/R injury. Fukuda et al. established a rat model of hepatic I/R in 2007 [34]. They found that hydrogen has very significant therapeutic effects on liver ischemic injury by hematoxylin and eosin (HE) staining, malondialdehyde (MDA), and enzyme test. In 2009, Kajiya et al. found that hydrogen has preventive effects on concanavalin-induced hepatitis [8]. In the same year, Tsai et al. found that drinking hydrogen-rich water can protect mice from carbon tetrachloride-induced liver damage [35]. Sun HY et al. demonstrated that hydrogen was effective not only on acute liver damage but also on cirrhosis in three kinds of liver injury model by using GalN/LPS, CCl4, and DEN [36]. Liu Q et al. found that intraperitoneal injection of hydrogen-rich saline could improve the antioxidant capacity of liver and inhibit hepatic inflammatory response, which has great clinical significance [37].

It has been reported that electrolytic water has protective effects on alcoholic liver injury which suggests that hydrogen has a sobering effect. Recent studies demonstrated that drinking hydrogen water for a long time can improve diet-induced fatty liver, not only improving liver function and liver morphology such as fibrosis, but also blocking cellular signaling pathways [38]. When fatty liver becomes a new lifestyle disease, the value of the hydrogen will be obvious. In this study, the authors also demonstrated that long-term consumption of hydrogen water can not

only improve fatty liver but also reduce the possibility of the occurrence of liver cancer significantly, which has a greater significance. It is the most direct evidence of hydrogen in the prevention of tumorigenesis.

Another research studied the therapeutic effects of hydrogen on obesity which is related to fatty liver [31]. The study is mainly focused on the hepatic metabolism of fat and proved that hydrogen could promote an important signaling molecule FGF21, which leads to weight loss and exerts therapeutic effects on fatty liver. There are more than 22 members in the family of fibroblast growth factor (FGF) that have a variety of biological activities. FGF21 is a new member of FGF family in the year 2000, which specifically expressed primarily in the liver, kidney, and adipose tissue. Studies have shown that FGF21 is a kind of very meaningful cytokines protecting glucose metabolism homeostasis. Its biological function is mainly reflected in the regulation of glucose and lipid metabolism which has a similar effect as insulin. FGF21 activator is known as a targeting molecule of the next-generation drug treating diabetes. Preliminary clinical studies have been reported that hydrogen has therapeutic effects on diabetes [31].

Many hydrogen product manufacturers have described the therapeutic effects of hydrogen on fatty liver which cannot be objective evidence, but also suggests its effects on the disease. The next step is focused on clinical observation of hydrogen on the disease.

There is only a few clinical research of hydrogen on liver disease that has been reported. Kang et al. performed a randomized placebo-controlled study of hydrogen water in 49 patients receiving radiation therapy for malignant liver tumors. Hydrogen improved the quality of life (QOL) scores. In particular, hydrogen efficiently prevented loss of appetite [39].

The studies above show that, as a selective antioxidant, hydrogen has significant therapeutic effects on hepatic ischemia, hepatitis, fatty liver, and other types of liver diseases, which indicate that hydrogen has very broad prospects on liver diseases.

6.3.3 The Outlook of Hydrogen in the Liver Diseases

Although the study of hydrogen in the liver disease has a certain size, there are still many blind spots. (1) Organ transplantation. Study of hydrogen in the liver transplantation is still a blank.

There are related studies of hydrogen on organ transplantation, including kidney, heart, small intestine, and lung. Although liver transplantation has been widely used in clinic, but there is no study about hydrogen on liver transplantation. By learning from other organ transplant injury research model, protective effects of hydrogen on liver transplantation are worth exploring. (2) Viral hepatitis. There is no report of hydrogen on various types of viral hepatitis research. Although it is hard to say hydrogen has direct antiviral effects, but virus-induced liver inflammation immune therapy can be an important basis for using hydrogen. (3) Clinical research. This is of course important and also a common problem existing in the field of hydrogen biology. Biological effects of hydrogen are lacking clinical research evidence,

especially those kinds of autoimmune disease, dermatitis, fatty liver, and obesity; chemical radiation therapy are very worthy to carry out clinical research. Nonalcoholic steatohepatitis and accompanying hepatocarcinogenesis (NASH) is one of the important liver diseases to overcome.

6.4 Therapeutic Effects of Hydrogen on Ophthalmic Diseases and Ear Diseases

6.4.1 Retina Ischemia-Reperfusion Injury

Hydrogen eye drops have been proved effective on retina I/R injury. The study showed potential and broad application prospects of hydrogen eye drops [40]. They prepared H_2-loaded eye drops (0.8 mM, pH 7.2) by dissolving H_2 gas into saline to saturated level and then administered the H_2-loaded eye drops to the ocular surface continuously (4 L/min) during the ischemia or reperfusion periods. The H_2-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 H_2 solution.

6.4.2 Erythematous Skin Diseases

Ono et al. treated four patients of acute erythematous skin diseases with fever and/or pain by H_2-enriched intravenous fluid [41]. All of the four patients received intravenous administration of 500 mL of H_2-enriched fluid in 30 min for more than 3 days except in one patient for only once. Erythema of these four patients and associated symptoms improved significantly after the H_2 treatment and did not recur.

6.4.3 Skin Tumors

Until 1975, Dole et al. [3] reported that hyperbaric hydrogen may be a possible treatment for cancer. They found a marked regression of the skin squamous cell carcinoma by inhalation of a mixture (2.5 % O_2 and 97.5 % H_2) at a total of 0.8 MPa for 2 weeks in a mouse model.

6.4.4 Radiation-Induced Skin Injury

Skin is a biological defense barrier of human body. Radiation injures skin directly by radiation energy or indirectly by free radicals, causing radiodermatitis which occurred in nearly 95 % of patients receiving radiation therapy [42]. There are

generally two types of radiodermatitis: acute radiodermatitis (usually occur within 90 days) and chronic radiodermatitis (may occurred over a prolonged period) which is often exhibited by the onset of erythema, swelling, blisters, and ulceration, followed by development of chronic inflammation, necrosis, fibrosis, and lymphedema [43, 44]. In 2012, Guo et al. [45] reported first that hydrogen-rich saline protected against ultraviolet B (UVB) radiation injury, possibly by reducing inflammation and oxidative stress. They demonstrated that hydrogen-rich saline had protective effects by altering the levels of markers, including necrosis factor alpha, IL-1â, IL-6, tissue superoxide dismutase, MDA, and nitric oxide activity, and relieved morphological skin injury against UVB radiation injury on C57BL/6 rats. In 2013, Mei et al. [42] reported the radioprotective effects of hydrogen on skin in vitro and vivo. In the study, our group found hydrogen significantly reduced the severity of dermatitis caused by radiation, accelerated tissue recovery, and reduced the extent of radiation-induced weight loss in rats after a single dose of 15 or 20 Gy radiation. We also found hydrogen protected rats from cumulative doses of 30 Gy delivered in three fractions. In the study, hydrogen also protected immortalized human keratinocytes (HaCaT cells) from radiation-induced injury. In 2013, Rosa Mistica Ignacio et al. [46] demonstrated the protective effect of hydrogen-reduced water (HRW) on UVB-mediated skin injury in hairless mice by balneotherapy. In their study, bathing with HRW significantly reduced the levels of skin damage by decreasing the level of inflammatory cytokines, including IL-1β, IL-6, TNF-α and IFN-γ, as well as increased activity of glutathione peroxidase.

In their study, HRW bathing also protect UV-induced corneocytes damage and ultrastructural changes.

Interestingly, in a recent study by Mi Hee Shin et al. [47], they observed that H(H_2O) (atomic hydrogen surrounded by water molecules) application to human skin prevented UV-induced erythema and DNA damage. In their study, H(H_2O) significantly prevented UV-induced MMP-1, COX-2, IL-6, and IL-1β mRNA expressions in human skin in vivo. They also found that H(H_2O) prevented UV-induced ROS generation and inhibited UV-induced MMP-1, COX-2, and IL-6 expressions, and UV-induced JNK and c-Jun phosphorylation in HaCaT cells.

6.5 Therapeutic Effects of Hydrogen on Respiratory Diseases

Inhaled therapeutic gas is a reasonable approach for the treatment of lung injury as imbalances between ROS and the antioxidant defense system are involved in certain pulmonary pathologic conditions, such as lung inflammation, ventilator-induced lung injury (VILI), and acute respiratory distress syndrome [48–51]. In 2010, Huang et al. showed that inhalation of 2% hydrogen gas attenuates VILI in a mouse model. Ventilation with 2% hydrogen in balanced air significantly ameliorated VILI-induced lung injury. This data strongly suggests that inhaled hydrogen gas could therapeutically mitigate VILI via its antioxidant and anti-inflammatory effects [52].

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. Hyperoxia in the presence of hydrogen significantly reduced hyperoxia-induced oxidative stress injury in premature rat type II alveolar epithelial cells, improved the cellular antioxidant capacity, stabilized the mitochondrial membrane potential, and reduced the inhibitory effect of hyperoxia on cell proliferation [53]. Acute lung injury secondary to remote organ damage and followed by deleterious systemic inflammation is a critical event. Mao et al. reported that hydrogen-rich saline treatment attenuated lung injury induced by intestinal I/R injury in a rat model [54]. The lung is one of the organs most susceptible to radiation injury [55]. Radiation pneumonitis is an inflammation of the lungs that occurs when lung or whole body was irradiated. Development of interstitial pneumonitis increases according to radiation dose, especially single-fraction total body irradiation at higher dose rates [56] and higher total lung doses [57, 58]. In 2011, our group hypothesized that hydrogen may be a possible prevention strategy for radiation pneumonitis [59]. In 2011, Terasaki et al. [60] showed that H_2 reduced irradiation-induced OH levels in human lung epithelial cell line A549 cells. They demonstrated that pretreatment of H_2 could reduce the fluorescence intensity of hydroxyphenyl fluorescein in irradiated A549 cells. They demonstrated pretreatment of H_2 reduced the products of oxidative stress, including 4-hydroxy-2-nonenal, 8-hydroxydeoxyguanosine, etc. H_2 could also significantly reduce levels of apoptosis-associated proteins, including Bax and active caspase 3 in irradiated A549 cells after a 24-h incubation with H_2-rich solution. In vivo, they demonstrated that H_2 treatment reduced oxidative stress and apoptosis, measures of acute damage in the lungs of mice within 1 week after whole thorax irradiation by immunohistochemistry and immunoblotting. In their study, H_2 treatment reduced lung fibrosis by chest computed tomography, Ashcroft scores, and type III collagen deposition at 5 months after irradiation.

6.6 Therapeutic Effects of Hydrogen on Inflammatory Diseases

6.6.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 1% of the population. It is characterized by irreversible joint disorder accompanied by destruction of bone and cartilage, which causes serious morbidity. ROS play a central role both upstream and downstream of NF-κB and TNF-α pathways, which are located at the center of the inflammatory response. Among the ROS, the hydroxyl radical is the most harmful, and molecular hydrogen (H_2) is a selective scavenger. It was demonstrated that consumption of water with a high concentration of molecular hydrogen significantly improves the disease activity and

reduces the oxidative stress in RA for this species [61, 62]. It has been shown that H_2 is useful when administered along with the conventional therapy in RA as it acts to reduce oxidative stress in the patients. Especially in the early stage, H_2 showed significant therapeutic potential, which also seemed to assist diagnosis and treatment decisions of RA.

6.6.2 Ulcerative Colitis

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that features a chronic, relapsing, and remitting inflammatory condition of the intestine, which is associated with enhanced production of ROS and altered angiogenesis. It has been reported that the treatment with hydrogen-rich saline reduced the weight loss and diarrhea and alleviated the colonic mucosal damage in the UC rats. In addition, the expression of vascular endothelial growth factor in the UC rats increased and could be inhibited by hydrogen treatment [63].

6.7 Studies of Hydrogen in Translational Medicine

Up to now , there have been seven kinds of diseases reported in clinical studies, including type II diabetes, metabolic syndrome, RA, hemodialysis, inflammation/mitochondrial muscle disease, brainstem ischemia, radiation therapy. In the World Health Organization Registration information, we found there are some clinical studies without published papers. By reading the following description of these studies, clinical researchers can learn more about the research progress of clinical studies of hydrogen.

Biological effects of hydrogen are mainly obtained in rodent models. So far, only seven preliminary clinical studies of hydrogen have been reported. These clinical studies are only preliminary clinical observation and insufficient to support hydrogen as a clinical treatment. Here we reviewed these studies. A hydrogen clinical trial in Parkinson's disease has been carried out which showed good therapeutic effects which has not been published (in fact, Japanese has a large clinical data about cognitive impairment and elderly diabetes). Type II diabetes, metabolic syndrome, and RA have been described above. Here, we describe the other four diseases.

6.7.1 Hemodialysis

An open-label placebo-controlled crossover trial of 12 sessions of hemodialysis in 8 patients and an open-label trial of 78 sessions of hemodialysis in 21 patients were performed by Nakayama et al. [25, 64]. In both studies, continuous sessions of hemodialysis with hydrogen-rich dialysis solution decreased systolic blood pressure

before and after dialysis. In the short-term study, plasma methylguanidine was significantly decreased. In the long-term study, plasma monocyte chemoattractant protein 1 and myeloperoxidase were significantly decreased.

6.7.2 Muscular Diseases

Ohno et al. performed an open-label trial of hydrogen water in 14 patients with muscular diseases, including muscular dystrophies, polymyositis/dermatomyositis, and mitochondrial myopathies, and a randomized, double-blind, placebo-controlled trial of hydrogen water or dehydrogenized water in 22 patients with dermatomyositis and mitochondrial myopathies [65]. In the open-label trial, significant improvements were observed in lactate-to-pyruvate ratio, fasting blood glucose, serum MMP-3, and triglycerides. Especially, the lactate-to-pyruvate ratio, which is a sensitive biomarker for the compromised mitochondrial electron transport system, was decreased by 28% in mitochondrial myopathies. In addition, MMP-3, which represents the activity of inflammation, was decreased by 27% in dermatomyositis. In the double-blind trial, a statistically significant improvement was observed only in serum lactate in mitochondrial myopathies, but lactate-to-pyruvate ratio in mitochondrial myopathies and MMP-3 in dermatomyositis were also decreased. Lack of statistical significance with the double-blind study was likely due to the shorter observation period and the lower amount of hydrogen compared to those of the open-label trial.

6.7.3 Side Effects of Radiation Therapy on Liver Cancer

A randomized placebo-controlled study of hydrogen water in 49 patients receiving radiation therapy for malignant liver tumors was performed by Kang et al. [39]. Hydrogen suppressed the elevation of total hydroperoxide levels, maintained serum antioxidant capacity, and improved the QOL scores. In particular, hydrogen efficiently prevented loss of appetite. Although the patients were randomly assigned to the hydrogen and placebo groups, the study could not be completely blinded because hydrogen was produced with a metallic magnesium stick, which generated hydrogen bubbles.

6.7.4 Brainstem Infarction

Edaravone is the only one antioxidant drug which has been approved to treat stroke. Ono et al. intravenously administered hydrogen along with edaravone in eight patients with acute brain stem infarction and compared MRI indices of 26 patients who received edaravone alone [13]. The MRI indices were all much more improved with the combined infusion of edaravone and hydrogen.

No adverse effect of hydrogen has been documented in the seven human diseases described above. Among the seven diseases, the most prominent effect was observed in subjects with metabolic syndrome, who consumed 1.5–2.0 L of hydrogen water per day. The amount of hydrogen water may be a critical parameter that determines clinical outcome. It is also interesting to note that lipid and glucose metabolisms were analyzed in three studies and all showed favorable responses to hydrogen [29, 32, 65]. This suggests a key regulatory role of hydrogen in the body fat metabolism and glucose metabolism.

6.8 Barriers in Studies on Biological Effects of Hydrogen

Although research on the biological effects of hydrogen is vigorous, there is no substantive progress in those studies after several years. Even some basic academic issues do not have clear answers.

There are two major problems or mysteries in the effects of hydrogen. First, dose–effect relationship was still unclear. No matter animal experiments or clinical observation, the dosage of hydrogen is small, but the effects are significant. Second, a large amount of hydrogen can be produced by gut bacteria in human and animals, but why the increase of such a small amount of hydrogen can generate such significant effects? In addition, other issues, such as the molecular basis of hydrogen, are the best way to use hydrogen for different diseases; the dose, frequency, etc. still need more research.

Although the biological effect of hydrogen has been demonstrated in more than 60 animal disease models and 6 human diseases, only two kinds of diseases, including cerebral infarction and metabolic syndrome, have both animal and clinical studies. Hydrogen has no side effects, which is a significant advantage to carry out clinical research. Some human diseases, including Parkinson's disease, are carried out which have achieved some good results. We believe that hydrogen will have certain therapeutic value for more human diseases. Of course, exploring the mechanism of hydrogen is very worthy and may be full of hardship.

References

1. Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med. 2007;13:688–94.
2. Ohta S. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. Pharmacol Ther. 2014. Oct;144(1):1-11.
3. Dole M, Wilson FR, Fife WP. Hyperbaric hydrogen therapy: a possible treatment for cancer. Science. 1975;190:152–4.
4. Gharib B, Hanna S, Abdallahi OM, Lepidi H, Gardette B, De Reggi M. Anti-inflammatory properties of molecular hydrogen: investigation on parasite-induced liver inflammation. C R Acad Sci III. 2001;324:719–24.

5. Matchett GA, Fathali N, Hasegawa Y, et al. Hydrogen gas is ineffective in moderate and severe neonatal hypoxia–ischemia rat models. Brain Res. 2009;1259:90–7.
6. Fujita R, Tanaka Y, Saihara Y, Yamakita M, Ando D, Koyama K. Effect of molecular hydrogen saturated alkaline electrolyzed water on disuse muscle atrophy in gastrocnemius muscle. J Phys Anthropol. 2011;30:195–201.
7. Powers SK, Smuder AJ, Judge AR. Oxidative stress and disuse muscle atrophy: cause or consequence? Curr Opin Clin Nutr Metab Care. 2012;15:240–5.
8. Kajiya M, Sato K, Silva MJ, et al. Hydrogen from intestinal bacteria is protective for Concanavalin A-induced hepatitis. Biochem Biophys Res Commun. 2009;386:316–21.
9. Cai J, Kang Z, Liu WW, et al. Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model. Neurosci Lett. 2008;441:167–72.
10. Kubota M, Shimmura S, Kubota S, et al. Hydrogen and N-acetyl-L-cysteine rescue oxidative stress-induced angiogenesis in a mouse corneal alkali-burn model. Invest Ophthalmol Vis Sci. 2011;52:427–33.
11. Li J, Wang C, Zhang JH, Cai JM, Cao YP, Sun XJ. Hydrogen-rich saline improves memory function in a rat model of amyloid-beta-induced Alzheimer's disease by reduction of oxidative stress. Brain Res. 2010;1328:152–61.
12. Wang C, Li J, Liu Q, et al. Hydrogen-rich saline reduces oxidative stress and inflammation by inhibit of JNK and NF-kappaB activation in a rat model of amyloid-beta-induced Alzheimer's disease. Neurosci Lett. 2011;491:127–32.
13. Ono H, Nishijima Y, Adachi N, et al. Improved brain MRI indices in the acute brain stem infarct sites treated with hydroxyl radical scavengers, Edaravone and hydrogen, as compared to Edaravone alone. A non-controlled study. Med Gas Res. 2011;1:12.
14. Liu Y, Liu W, Sun X, et al. Hydrogen saline offers neuroprotection by reducing oxidative stress in a focal cerebral ischemia-reperfusion rat model. Med Gas Res. 2011;1:15.
15. Fujita K, Seike T, Yutsudo N, et al. Hydrogen in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. PloS one. 2009;4:e7247.
16. Fu Y, Ito M, Fujita Y, et al. Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. Neurosci Lett. 2009;453:81–5.
17. Yoritaka A, Takanashi M, Hirayama M, Nakahara T, Ohta S, Hattori N. Pilot study of H(2) therapy in Parkinson's disease: a randomized double-blind placebo-controlled trial. Mov Disord. 2013;28:836–9.
18. Shen L, Wang J, Liu K, et al. Hydrogen-rich saline is cerebroprotective in a rat model of deep hypothermic circulatory arrest. Neurochem Res. 2011;36:1501–11.
19. Jiao L, Zhang J, Li Z, Liu H, Chen Y, Xu S. Edaravone alleviates delayed neuronal death and long-dated cognitive dysfunction of hippocampus after transient focal ischemia in Wistar rat brains. Neurosci. 2011;182:177–83.
20. Zhan Y, Chen C, Suzuki H, Hu Q, Zhi X, Zhang JH. Hydrogen gas ameliorates oxidative stress in early brain injury after subarachnoid hemorrhage in rats. Crit Care Med. 2012;40:1291–6.
21. Hong Y, Guo S, Chen S, Sun C, Zhang J, Sun X. Beneficial effect of hydrogen-rich saline on cerebral vasospasm after experimental subarachnoid hemorrhage in rats. J Neurosci Res. 2012;90:1670–80.
22. Takeuchi S, Wada K, Nagatani K, Osada H, Otani N, Nawashiro H. Hydrogen may inhibit collagen-induced platelet aggregation: an ex vivo and in vivo study. Intern Med. 2012;51:1309–13.
23. Chen CH, Manaenko A, Zhan Y, et al. Hydrogen gas reduced acute hyperglycemia-enhanced hemorrhagic transformation in a focal ischemia rat model. Neuroscience. 2010;169:402–14.
24. Schapira AH. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. Lancet Neurol. 2008;7:97–109.
25. Nakayama M, Nakano H, Hamada H, Itami N, Nakazawa R, Ito S. A novel bioactive haemodialysis system using dissolved dihydrogen (H2) produced by water electrolysis: a clinical trial. Nephrol Dial Transplant. 2010;25:3026–33.

26. Jucker M, Walker LC. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. Ann Neurol. 2011;70:532–40.
27. Nagata K, Nakashima-Kamimura N, Mikami T, Ohsawa I, Ohta S. Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent learning tasks during chronic physical restraint in mice. Neuropsychopharmacology. 2009;34:501–8.
28. Gu Y, Huang CS, Inoue T, et al. Drinking hydrogen water ameliorated cognitive impairment in senescence-accelerated mice. J Clin Biochem Nutr. 2010;46:269–76.
29. Kajiyama S, Hasegawa G, Asano M, et al. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutr Res. 2008;28:137–43.
30. Li Y, Hamasaki T, Nakamichi N, et al. Suppressive effects of electrolyzed reduced water on alloxan-induced apoptosis and type 1 diabetes mellitus. Cytotechnology. 2011;63:119–31.
31. Kamimura N, Nishimaki K, Ohsawa I, Ohta S. Molecular hydrogen improves obesity and diabetes by inducing hepatic FGF21 and stimulating energy metabolism in db/db mice. Obesity. 2011;19:1396–403.
32. Nakao A, Toyoda Y, Sharma P, Evans M, Guthrie N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome-an open label pilot study. J Clin Biochem Nutr. 2010;46:140–9.
33. Doyle MR, Davis SJ, Bastow RM, et al. The ELF4 gene controls circadian rhythms and flowering time in Arabidopsis thaliana. Nature. 2002;419:74–7.
34. Fukuda K, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S. Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. Biochem Biophys Res Commun. 2007;361:670–4.
35. Tsai CF, Hsu YW, Chen WK, et al. Hepatoprotective effect of electrolyzed reduced water against carbon tetrachloride-induced liver damage in mice. Food Chem Toxicol. 2009;47:2031–6.
36. Sun H, Chen L, Zhou W, et al. The protective role of hydrogen-rich saline in experimental liver injury in mice. J Hepatol. 2011;54:471–80.
37. Liu Q, Shen WF, Sun HY, et al. Hydrogen-rich saline protects against liver injury in rats with obstructive jaundice. Liver Int. 2010;30:958–68.
38. Kawai D, Takaki A, Nakatsuka A, et al. Hydrogen-rich water prevents progression of non-alcoholic steatohepatitis and accompanying hepatocarcinogenesis in mice. Hepatology. 2012;56:912–21.
39. Kang KM, Kang YN, Choi IB, et al. Effects of drinking hydrogen-rich water on the quality of life of patients treated with radiotherapy for liver tumors. Med Gas Res. 2011;1:11.
40. Oharazawa H, Igarashi T, Yokota T, et al. Protection of the retina by rapid diffusion of hydrogen: administration of hydrogen-loaded eye drops in retinal ischemia-reperfusion injury. Invest Ophthalmol Vis Sci. 2010;51:487–92.
41. Ono H, Nishijima Y, Adachi N, et al. Hydrogen(H2) treatment for acute erythymatous skin diseases. A report of 4 patients with safety data and a non-controlled feasibility study with H2 concentration measurement on two volunteers. Med Gas Res. 2012;2:14.
42. Mei K, Zhao S, Qian L, Li B, Ni J, Cai J. Hydrogen protects rats from dermatitis caused by local radiation. J Dermatolog Treat. 2014 Apr;25(2):182-8.
43. Murphy BA, Gilbert J. Dysphagia in head and neck cancer patients treated with radiation: assessment, sequelae, and rehabilitation. Semin Radiat Oncol. 2009;19:35–42.
44. Hymes SR, Strom EA, Fife C. Radiation dermatitis: clinical presentation, pathophysiology, and treatment 2006. J the Am Acad Dermatol. 2006;54:28–46.
45. Guo Z, Zhou B, Li W, Sun X, Luo D. Hydrogen-rich saline protects against ultraviolet B radiation injury in rats. J Biomed Res. 2012 Sep;26:365–71.
46. Ignacio RM, Yoon Y-S, Sajo MEJ, et al. The balneotherapy effect of hydrogen reduced water on UVB-mediated skin injury in hairless mice. Mol Cell Toxicol. 2013;9:15–21.
47. Shin MH, Park R, Nojima H, Kim HC, Kim YK, Chung JH. Atomic hydrogen surrounded by water molecules, H(H2O)m, modulates basal and UV-induced gene expressions in human skin in vivo. PloS one. 2013 Apr 24;8(4):e61696.

48. Nakamura T, Henson PM, Murphy RC. Occurrence of oxidized metabolites of arachidonic acid esterified to phospholipids in murine lung tissue. Anal Biochem. 1998;262:23–32.
49. Yoshimi N, Ikura Y, Sugama Y, et al. Oxidized phosphatidylcholine in alveolar macrophages in idiopathic interstitial pneumonias. Lung. 2005;183:109–21.
50. Whidden MA, McClung JM, Falk DJ, et al. Xanthine oxidase contributes to mechanical ventilation-induced diaphragmatic oxidative stress and contractile dysfunction. J Appl Physiol. 2009;106:385–94.
51. Bem RA, van Woensel JB, Bos AP, et al. Mechanical ventilation enhances lung inflammation and caspase activity in a model of mouse pneumovirus infection. Am J Physiol Lung Cell Mol Physiol. 2009;296:L46–56.
52. Huang CS, Kawamura T, Lee S, et al. Hydrogen inhalation ameliorates ventilator-induced lung injury. Crit Care. 2010;14:R234.
53. Yao L, Xu F, Luo C, et al. [Protective effect of hydrogen against hyperoxia-induced type II alveolar epithelial cell injury]. Nan Fang Yi Ke Da Xue Xue Bao. 2013;33:193–6.
54. Mao YF, Zheng XF, Cai JM, et al. Hydrogen-rich saline reduces lung injury induced by intestinal ischemia/reperfusion in rats. Biochem Biophys Res Commun. 2009;381:602–5.
55. Para AE, Bezjak A, Yeung IW, Van Dyk J, Hill RP. Effects of genistein following fractionated lung irradiation in mice. Radiother Oncol. 2009;92:500–10.
56. Deeg HJ. Acute and delayed toxicities of total body irradiation. Seattle Marrow Transplant Team. Int J Radiat Oncol Biol Phys. 1983;9:1933–9.
57. Beyzadeoglu M, Oysul K, Dirican B, et al. Effect of dose-rate and lung dose in total body irradiation on interstitial pneumonitis after bone marrow transplantation. Tohoku J Exp Med. 2004;202:255–63.
58. Sampath S, Schultheiss TE, Wong J. Dose response and factors related to interstitial pneumonitis after bone marrow transplant. Int J Radiat Oncol Biol Phys. 2005;63:876–84.
59. Chuai Y, Zhao L, Ni J, et al. A possible prevention strategy of radiation pneumonitis: combine radiotherapy with aerosol inhalation of hydrogen-rich solution. Med Sci Monit. 2011 Apr;17(4):HY1-4.
60. Terasaki Y, Ohsawa I, Terasaki M, et al. Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress. Am J Physiol Lung Cell Mol Physiol. 2011 Oct;301:L415–26.
61. Ishibashi T, Sato B, Rikitake M, et al. Consumption of water containing a high concentration of molecular hydrogen reduces oxidative stress and disease activity in patients with rheumatoid arthritis: an open-label pilot study. Med Gas Res. 2012;2:27.
62. Ishibashi T, Sato B, Shibata S, et al. Therapeutic efficacy of infused molecular hydrogen in saline on rheumatoid arthritis: a randomized, double-blind, placebo-controlled pilot study. Int Immunopharmacol. 2014;21:468–73.
63. He J, Xiong S, Zhang J, et al. Protective effects of hydrogen-rich saline on ulcerative colitis rat model. J Surg Res. 2013;185:174–81.
64. Nakayama M, Kabayama S, Nakano H, et al. Biological effects of electrolyzed water in hemodialysis. Nephron Clin Pract. 2009;112:c9–15.
65. Ito M, Ibi T, Sahashi K, Ichihara M, Ito M, Ohno K. Open-label trial and randomized, double-blind, placebo-controlled, crossover trial of hydrogen-enriched water for mitochondrial and inflammatory myopathies. Med Gas Res. 2011;1:24.