

Recent Progress Toward Hydrogen Medicine: Potential of Molecular Hydrogen for Preventive and Therapeutic Applications

Shigeo Ohta*

Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School

Abstract: Persistent oxidative stress is one of the major causes of most lifestyle-related diseases, cancer and the aging process. Acute oxidative stress directly causes serious damage to tissues. Despite the clinical importance of oxidative damage, antioxidants have been of limited therapeutic success. We have proposed that molecular hydrogen (H_2) has potential as a “novel” antioxidant in preventive and therapeutic applications [Ohsawa *et al.*, Nat Med. 2007; 13; 688-94]. H_2 has a number of advantages as a potential antioxidant: H_2 rapidly diffuses into tissues and cells, and it is mild enough neither to disturb metabolic redox reactions nor to affect reactive oxygen species (ROS) that function in cell signaling, thereby, there should be little adverse effects of consuming H_2 . There are several methods to ingest or consume H_2 , including inhaling hydrogen gas, drinking H_2 -dissolved water (hydrogen water), taking a hydrogen bath, injecting H_2 -dissolved saline (hydrogen saline), dropping hydrogen saline onto the eye, and increasing the production of intestinal H_2 by bacteria. Since the publication of the first H_2 paper in *Nature Medicine* in 2007, the biological effects of H_2 have been confirmed by the publication of more than 38 diseases, physiological states and clinical tests in leading biological/medical journals, and several groups have started clinical examinations. Moreover, H_2 shows not only effects against oxidative stress, but also various anti-inflammatory and anti-allergic effects. H_2 regulates various gene expressions and protein-phosphorylations, though the molecular mechanisms underlying the marked effects of very small amounts of H_2 remain elusive.

Keywords: Anti-inflammation, antioxidant, hydrogen medicine, medical gas, mitochondria, oxidative stress, ischemia-reperfusion, ROS.

1. INTRODUCTION

Oxidative stress arises from the strong cellular oxidizing potential of excess reactive oxygen species (ROS) [1]. Acute oxidative stress arises from a variety of situations, including ischemia reperfusion [2]. Persistent oxidative stress is widely accepted as one of the causes of most lifestyle-related diseases, cancer and the aging process [3-7]; however, many antioxidant supplements could not prevent cancer, myocardial infarction and atherosclerosis, but rather conversely increase mortality [8-11]; thus, it is very important to be aware of side effects when developing an effective antioxidant for the prevention of oxidative stress-related diseases.

We found that molecular hydrogen (H_2) has roles as a “novel” antioxidant in preventive and therapeutic applications [12]. H_2 has advantages as a potential antioxidant without adverse effects: it is mild enough neither to disturb metabolic redox reactions nor to affect ROS, which function in cell signaling [13-15] and has favorable distribution characteristics in its own physical ability to penetrate biomembranes and diffuse through barriers into cellular components.

Here, we review the recent progress toward therapeutic and preventive applications of H_2 in widespread fields.

2. ROS AS ONE OF THE MAJOR CAUSES OF ACUTE AND CHRONIC DISEASES

2.1. Persistent Oxidative Stress

ROS are generated inside the body throughout our daily lives, such as during hard exercise, smoking, exposure to ultraviolet rays or air pollution, aging, physical or psychological stress, and so on [16-19]. Inside every aerobic organism, ROS are generated when breathing consumes oxygen.

As the first step in generating persistent ROS, the majority of superoxide anion radicals ($\bullet O_2^-$) are generated in mitochondria by electron leakage from the electron transport chain [3, 7, 20, 21].

Superoxide dismutase converts to hydrogen peroxide (H_2O_2), which is metabolized by glutathione peroxidase or catalase to generate water (H_2O). Highly reactive hydroxyl radicals ($\bullet OH$) are generated from H_2O_2 via the Fenton or Weis reaction in the presence of catalytically active metals, such as Fe^{2+} and Cu^+ [22]; therefore, manipulation of the genes involved in anti-oxidation prolonged the lifespan or prevented disease models [23-27].

These ROSs are generated under the condition of excessively high membrane potential to leak electrons from the electron transport chain [28]. In fact, uncoupling proteins control the membrane potential to suppress the production of ROS and then consequently to repress diabetes [29-31].

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) functions as a protector against oxidative stress by detoxifying cytotoxic aldehydes, such as 4-hydroxy-2-nonenal [4, 5, 32]. Thus, a defect of ALDH2 sufficiently induces phenotypes of age-dependent dementia by accumulating such cytotoxic aldehydes [32]. Paradoxically, such aldehydes stimulate protective systems against oxidative stress [33]. Thus, oxidative stress has two faces, to damage tissues and to enhance protective systems.

2.2. Acute Oxidative Stress

Acute oxidative stress arises from various different situations: inflammation, cardiac or cerebral infarction, organ transplantation, heavy exercise, cessation of operative bleeding, and others [2, 34, 35]. In many cases, ischemia reperfusion is a critical cause to raise acute oxidative stress. In myocardial infarction, the accelerated generation of ROS by reperfusion of the ischemic myocardium is a potential mediator of reperfusion injury [36-39]. During myocardial reperfusion, $\bullet O_2^-$ is generated within the injured mitochondria via electron leakage from the electron transport chain. $\bullet O_2^-$ converts to H_2O_2 , and highly reactive $\bullet OH$ is generated from H_2O_2 as mentioned [22, 40].

These ROS mediate myocardial injury by inducing mitochondrial permeability transition pore (PTP) opening, causing a loss of mitochondrial membrane potential, and leading to mitochondrial swelling with membrane rupture [41]. Many attempts have been made to inhibit ROS production to limit the extent of reperfusion

*Address correspondence to this author at the 1-396, Kosugi-machi, Nakahara-ku, Kawasaki city, Kanagawa pref. 211-8533 Japan; Tel: +81-44-733-9267; Fax: +81-44-733-9268; E-mail: ohta@nms.ac.jp

injury. The administration of ROS scavengers at the time of reperfusion has produced conflicting results that can be partially explained by the dual role of ROS in ischemia-reperfusion hearts [42, 43]. The majority of detrimental effects associated with lethal reperfusion injury are attributed to $\bullet\text{OH}$. By comparison, $\bullet\text{O}_2^-$ and H_2O_2 have less oxidative energy and, paradoxically, are implicated as crucial signaling components in the establishment of tolerance to oxidative stress [44, 45]. Thus, cytotoxic radicals such as $\bullet\text{OH}$ must be neutralized without compromising the essential biological activities of other ROS, including $\text{NO}\bullet$ [46, 47].

3. CHARACTERISTICS OF MOLECULAR HYDROGEN

We found that H_2 functions as a mild but effective antioxidant [12]. Hydrogen is the most abundant element in the universe, constituting nearly 75% of the universe's mass; however, hydrogen is absent on the earth in its monoatomic form and is present in water and organic or inorganic compounds. Hydrogen gas, with the molecular formula H_2 , is a colorless, odorless, tasteless and highly combustible diatomic gas. The earth's atmosphere contains less than 1 part per million of hydrogen gas [48].

H_2 is rather less active and behaves as an inert gas in the absence of catalysts or at body temperature. H_2 does not react with most compounds, including oxygen gas at room temperature. Hydrogen gas is flammable only at temperature higher than 527°C , and explodes by a rapid chain reaction with oxygen only in the explosive range of the H_2 concentration (4 - 75%, vol/vol).

H_2 can be dissolved in water up to 0.8 mM (1.6 ppm, wt/vol) under atmospheric pressure, and rapidly H_2 penetrates the glass and plastic walls of any vessels, while aluminum containers are able to retain hydrogen gas for a long time.

4. SCAVENGING EFFECTS ON HYDROXYL RADICALS IN CULTURED CELLS

4.1. Scavenging $\bullet\text{OH}$, but Not $\bullet\text{O}_2^-$, H_2O_2 and NO in Cultured Cells

H_2 scavenges $\bullet\text{OH}$, but not $\bullet\text{O}_2^-$, H_2O_2 and NO in cultured cells. H_2 was dissolved in culture medium under high pressure of hydrogen gas or by simply bubbling with hydrogen gas. The medium was combined with O_2 -saturated medium at the ratio of 8 : 2 (H_2 : O_2). Hydrogen and oxygen concentrations and pH were monitored with each specific electrode. Cultured cells were treated with a mitochondrial respiratory complex III inhibitor, antimycin, A to induce excess $\bullet\text{O}_2^-$ production. Following such treatment, $\bullet\text{O}_2^-$ was rapidly converted to H_2O_2 and then $\bullet\text{OH}$. The addition of antimycin A actually increased levels of $\bullet\text{O}_2^-$ and H_2O_2 inside cells; however, H_2 dissolved in culture medium did not change their levels. Additionally, H_2 did not decrease the steady-state level of NO in cells. In contrast, H_2 treatment significantly decreased levels of $\bullet\text{OH}$, as judged by the decrease in the fluorescent signal of hydroxyphenyl fluorescein (HPF) [49] and in the spin trap signals. Notably, H_2 decreased $\bullet\text{OH}$ levels even in the nuclear region [12].

After antimycin A treatment, H_2 prevented the decline of the mitochondrial membrane potential. This suggested that H_2 protected mitochondria from $\bullet\text{OH}$. Along with this protective effect, H_2 also prevented a decrease in the cellular level of ATP synthesized in mitochondria. The fact that H_2 protected mitochondria and nuclear DNA provided evidence that H_2 penetrated most membranes and diffused into organelles. Consequently, H_2 protected cultured cells against oxidative stress [12].

4.2. Other Effects Shown by Using Culture Systems

H_2 dissolved in medium protected cultured auditory hair cells from free radicals [50] and is suggested to decrease $\bullet\text{OH}$, as judged by the decrease in HPF fluorescence in vestibular tissue [51].

$\bullet\text{OH}$ causes most ionizing radiation-induced cellular damage. H_2 exhibited protective effects against radiation-induced damage in

cultured cells and mice [52]. Cosmic radiation is known to induce DNA and lipid damage associated with increased oxidative stress and remains a major concern in space travel. It is expected that space mission activities will increase in coming years both in number and duration. It is therefore important to estimate and prevent the risks encountered by astronauts due to oxidative stress prior to developing clinical symptoms of disease. Schoenfeld *et al.* hypothesized that H_2 administration to astronauts by either inhalation or drinking hydrogen water may potentially yield a novel and feasible preventative/therapeutic strategy to prevent radiation-induced adverse events [53].

On the other hand, H_2 treatment prolonged the replicable lifespan of bone marrow multipotential stromal cells *in vitro* while preserving differentiation and paracrine potentials. Cell therapy with bone marrow multipotential stromal cells/mesenchymal stem cells represents a promising approach in the field of regenerative medicine. Low frequency of mesenchymal stem cells in adult bone marrow necessitates *ex vivo* expansion of mesenchymal stem cells after harvest; however, such manipulation causes cellular senescence with loss of differentiation, proliferative, and therapeutic potentials of mesenchymal stem cells. As oxidative stress is one of the key insults promoting cell senescence *in vivo* as well as *in vitro*, H_2 prevented the senescent process during mesenchymal stem cell expansion. Notably, 3% hydrogen gas treatment did not decrease $\bullet\text{OH}$, protein carbonyl, and 8-hydroxydeoxyguanosine, suggesting that scavenging $\bullet\text{OH}$ might not be responsible for these effects of hydrogen gas in this study [54].

5. ADVANTAGES OF HYDROGEN

5.1. Rapid Diffusion

H_2 has a number of advantages as a potential antioxidant. First, it has favorable distribution characteristics with its own physical ability to penetrate biomembranes and diffuse into the cytosol.

Excessive oxidative damage is a major factor because the mitochondrial respiratory chain is a significant source of damaging reactive oxygen species; however, despite the clinical importance of mitochondrial oxidative damage, antioxidants have been of limited therapeutic success. This may be because antioxidants are not selectively taken up by mitochondria [55-57]. As H_2 effectively reaches the nucleus and mitochondria, the protection of nuclear DNA and mitochondria suggests preventive effects on lifestyle-related diseases, cancer and the aging process [12]. Moreover, H_2 passes through the blood brain barrier, although most antioxidant compounds cannot; this is also an advantage of H_2 .

Monitoring H_2 concentration inside various tissues can prove gaseous diffusion [58].

5.2. No Direct Elimination of Functionally Important ROS

Despite their cytotoxic effects, low concentrations of ROS, such as $\bullet\text{O}_2^-$ and H_2O_2 , function as signaling molecules and regulate apoptosis, cell proliferation, and differentiation [14, 15]. As mentioned, unexpectedly and notably, recent studies have suggested that excessive antioxidants increased mortality and rates of cancer [9, 11, 59-62] because they may interfere with some essential defensive mechanisms [13, 60, 63-67]. At higher concentrations, H_2O_2 is converted to hypochlorous acid by myeloperoxidase to defend against bacterial invasion [68]. Additionally, NO functions as a neurotransmitter and is essential for the dilation of blood vessels [69].

Since H_2 reduces $\bullet\text{OH}$ but does not affect $\bullet\text{O}_2^-$ and H_2O_2 having physiological roles [12], we propose that the adverse effects of H_2 are very small compared to other antioxidants.

5.3. No toxicity Even at Higher Concentration.

Several medical gasses are expected to provide more effective therapeutic interventions and preventive medicine despite their

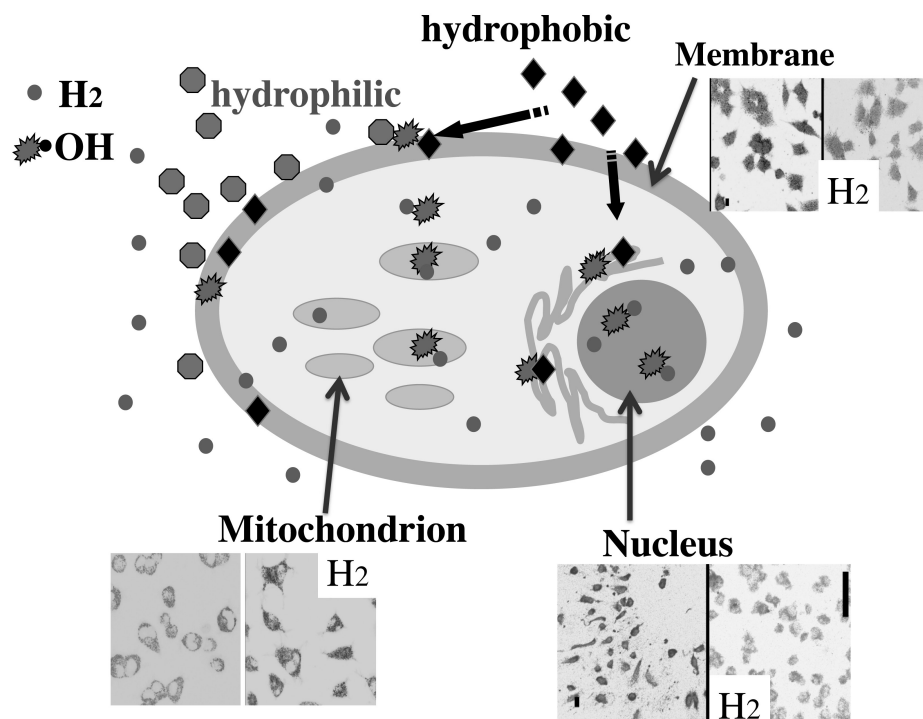


Fig. (1). Illustration of gaseous diffusion of H₂ in a cell. Most hydrophilic compounds (●) retain at membranes and cannot reach the cytosol, whereas most hydrophobic ones (◆) cannot penetrate biomembranes in the absence of specific carriers. In contrast, H₂ (●) can rapidly distribute into cytosol and organelles. PC12 cells were placed in culture media containing H₂ (0.6 mM) and O₂ (0.24 mM), and then oxidative stress was induced by adding antimycin A (10 µg/mL), an inhibitor of the electron transport chain of mitochondria, and maintained for 1 day. Two markers of oxidative stress were detected by immunostaining with anti-8-hydroxy-Guanine (**Nucleus**) and anti-4-hydroxy-2-nonenal (**Membrane**). Thirty minutes after adding antimycin A with or without H₂, 100 nM tetramethylrhodamine methyl ester (TMRM), a fluorescent detector of the membrane potential of **mitochondrion**, were added, incubated for 10 min, and cells were imaged with a laser scanning confocal microscope. These results indicate that H₂ reach the nucleus and mitochondria and protects them.

severe toxicity. Gas inhalation as disease therapy has received recent interest [70]. In past decades, there has been extraordinary, rapid growth in our knowledge of gaseous molecules, including nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H₂S), which have been known to play important roles in biological systems [71, 72].

In pre-clinical experimental models of disease, including ischemia-reperfusion injury, the inhalation of exogenous CO or H₂S has produced a favorable outcome for most vital organs [73-76]. In particular, NO has been approved as a therapeutic agent in clinical practice [77]. The inherent toxicity of these gasses must be investigated for gas inhalation to be considered an effective therapeutic strategy because these gasses are highly toxic at considerable concentrations. Additionally, NO enhances oxidative stress via the reaction with $\bullet\text{O}_2^-$ by the production of highly oxidative peroxynitrite ($\text{NO} + \bullet\text{O}_2^- \rightarrow \text{ONOO}^-$). It is unknown if the therapeutically effective threshold for CO or H₂S can be attained locally in target organs without delivering a potentially toxic level of the gasses via the lungs.

In contrast, H₂ has more advantages from the aspect of toxicity: H₂ has no cytotoxicity even at high concentration [78-81]. Furthermore, safety standards have been established for high concentrations of hydrogen gas for inhalation since high pressure hydrogen gas is used in deep diving gas mixes to prevent decompression sickness and arterial gas thrombi [81]. The safety of H₂ for humans is demonstrated by its application in Hydrellox, an exotic, breathing gas mixture of 49% H₂, 50% helium and 1% O₂, which is used to prevent decompression sickness and nitrogen narcosis during very deep technical diving [78-81].

6. METHODS OF INGEST HYDROGEN I: INHALATION OF HYDROGEN GAS

6.1. Inhalation of Hydrogen Gas

Inhalation of hydrogen gas is a straightforward therapeutic method. Hydrogen gas can be inhaled by delivering hydrogen gas through a ventilator circuit, facemask or nasal cannula. Since inhaled hydrogen gas acts more rapidly, it may be suitable for defense against acute oxidative stress. In particular, inhalation of gas does not affect blood pressure [12]; blood pressure increased by infusion may cause serious obstacles during the treatment of myocardial infarction. Hydrogen gas poses no risk of explosion in air and in pure oxygen when present at concentrations < 4%, as mentioned earlier; however, safety could be a concern and the desired concentration of H₂ must be monitored and maintained with an approved and commercially available tool.

Rats inhaled hydrogen gas in a mix of nitrous oxide (N₂O) (for anesthesia), O₂, and N₂. The inhalation of H₂ actually increased H₂ dissolved in arterial blood depending upon the hydrogen gas concentrations, and H₂ levels in venous blood were lower than in arterial blood; the different level between arterial and venous blood indicates the amount of H₂ incorporated into tissues [12].

6.2. Direct Demonstration of Rapid Diffusion of Hydrogen as a Medical Gas

Gasses possess the ability to diffuse readily in different materials and become uniformly distributed within a defined space. "Biologic gasses" are assumed to diffuse freely across biologic membranes, acting in a variety of functional capacities [70]; hydrogen gas is an example of this.

The gaseous diffusion of H₂ is indeed proven by monitoring its concentration inside various tissues. H₂ can be detected with specific electrodes. H₂ concentration has been monitored within a rat myocardium. The electrode was inserted into the non-ischemic myocardium of the left ventricle. The incremental rate of H₂ saturation for the non-ischemic myocardium and arterial blood was similar. Then, the electrode was inserted into the 'at risk' area for infarction to investigate the diffusion of H₂ into the ischemic myocardium, induced by coronary artery occlusion. Notably, H₂ concentration was increased even in the ischemic myocardium. Although the incremental rate of H₂ saturation was slower in the ischemic myocardium than in the non-ischemic myocardium, the peak level of H₂ in the ischemic myocardium was approximately two thirds of the value observed for the non-ischemic myocardium [58].

6.3. Protective Effects on Ischemia Reperfusion Model by Rat Cerebral Infarction

Hydrogen gas was applied to a rat model of ischemia-reperfusion as an acute model [82]. We produced focal ischemia by occlusion of the rat middle cerebral artery with subsequent reperfusion. One day after middle cerebral artery occlusion, infarct volumes decreased in a H₂-dependent manner. One week after middle cerebral artery occlusion, the difference in infarct volumes between non-treated and H₂-treated rats increased. H₂-treated rats also showed improvements in body weight and temperature and movement defects vs. untreated rats. Thus, H₂ suppressed not only the initial brain injury, but also the progression of injury. H₂ markedly decreased several oxidative stress markers. In this experiment, H₂ was demonstrated to have the potential to markedly decrease oxidative stress and suppress brain injury [12].

6.4. Protective Effects on Hepatic and Cardiac Ischemia Reperfusion Injury

Next, inhalation of hydrogen gas was also applied to a hepatic ischemia reperfusion injury model [83]. Inhalation of H₂ clearly attenuated the degeneration induced by hepatic ischemia reperfusion and increased the protective effect in an H₂-dependent manner. In contrast, helium gas (He) exhibited no effect, indicating that H₂ clearly has a specific protective effect [84].

The degree of cardioprotection against ischemia-reperfusion injury was evaluated by measuring oxidative damage and infarct size after left anterior descending coronary artery occlusion and reperfusion. Inhalation of an incombustible level of hydrogen gas (2%) before reperfusion significantly reduced oxidative stress-induced myocardial injury and infarct size without affecting hemodynamic parameters, and thereby prevented deleterious left ventricle remodeling [58].

6.5. Protective Effects in Organ Transplantation

H₂ inhalation significantly ameliorated intestinal and pulmonary transplant injury and prevented remote organ inflammation via its antioxidant effects [85, 86]. Ischemia/reperfusion injury during small intestinal and lung transplantation frequently causes complications, including dysmotility, inflammation and organ failure.

H₂ treatment resulted in significantly improved gastrointestinal transit, as well as jejunal smooth muscle contractility in response to bethanechol [86]. Graft lipid peroxidation was significantly reduced in the presence of H₂, demonstrating antioxidant effects of H₂ in the transplanted lungs. Exposure to 2% hydrogen gas significantly blocked the production of several pro-inflammatory mediators and reduced apoptosis with induction of the anti-apoptotic molecules B-cell lymphoma-2 and B-cell lymphoma-extra large.

Rat cardiac cold ischemia reperfusion injury was ameliorated with inhaled H₂ or carbon monoxide (CO), or both. Combined therapy with H₂ and CO demonstrated enhanced therapeutic efficacy via both anti-oxidant and anti-inflammatory mechanisms, and may be a clinically feasible approach for preventing cold ischemia reper-

fusion injury of the myocardium [87]. Inhaled hydrogen gas effectively reduced ventilator-induced lung injury-associated inflammatory responses, at both a local and systemic level, via its antioxidant, anti-inflammatory and anti-apoptotic effects [88].

6.6. Protective Effects in Infectious Diseases and anti-inflammatory Effects

Sepsis, a multiple organ dysfunction syndrome, is the leading cause of death in critically ill patients [89]. Hydrogen gas inhalation significantly improved the survival rate and organ damage of septic mice with moderate or severe cecal ligation and puncture by reducing levels of early and late pro-inflammatory cytokines in serum and tissues [90].

The effects of 2% H₂ treatment was investigated on the survival rate and organ damage in zymosan-induced generalized inflammation model. The beneficial effects of H₂ treatment zymosan-induced organ damage were associated with decreased levels of oxidative product, increased activities of antioxidant enzyme, and reduced levels of early and late pro-inflammatory cytokines in serum and tissues. H₂ treatment protected against multiple organ damage in a zymosan-induced generalized inflammation model, suggesting the potential use of H₂ as a therapeutic agent in the therapy of conditions associated with inflammation-related multiple organ dysfunction syndrome [91].

6.7. Others

Other reports had the following titles: Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model [92]; hydrogen gas reduced acute hyperglycemia-enhanced hemorrhagic transformation in a focal ischemia rat model [93]; hydrogen is neuroprotective and preserves cerebrovascular reactivity in asphyxiated newborn pigs [94]; beneficial effects of hydrogen gas in a rat model of traumatic brain injury via reducing oxidative stress [95]; beneficial effects of hydrogen gas against spinal cord ischemia-reperfusion injury in rabbits [96]; and hydrogen protects vestibular hair cells from free radicals [97].

7. METHODS OF INGEST HYDROGEN II: ORAL INGESTION OF HYDROGEN WATER

7.1. Oral Ingestion by Drinking Hydrogen Water

Since inhaled hydrogen gas acts more rapidly, it may be suitable for defense against acute oxidative stress. In particular, inhalation of gas does not affect blood pressure; blood pressure increased by infusion may be serious in myocardial infarction; however, inhalation of hydrogen gas may be unsuitable or not practical as continuous H₂ consumption in daily life for preventive use. In contrast, solubilized H₂ (H₂-dissolved water; namely, hydrogen water) may be beneficial since it is a portable, easily administered and a safe means of delivering H₂ [98]. H₂ can be dissolved in water up to 0.8 mM under atmospheric pressure at room temperature as mentioned earlier. Unexpectedly, drinking hydrogen water had effects comparable to hydrogen gas inhalation [99].

Hydrogen water can be made by several methods, including dissolving hydrogen gas in water under high pressure, dissolving electrolyzed H₂ in water, and by the reaction of magnesium metal with water. The method of dissolving hydrogen gas under high pressure has an advantage because it is applicable not only using water but also any other solvents.

When water saturated with H₂ was placed into the stomach of a rat, H₂ was detected at several μ M level in blood [98, 99]. Moreover, hepatic H₂ was monitored with a needle-type hydrogen electrode, and H₂ accumulated after oral administration of hydrogen water, partly explaining why consumption of even a small amount of H₂ over a short dwell time could efficiently improve various disease models. An additional *in vitro* experiment confirmed that

polymers of carbohydrates, including glycogen and starch, have an affinity for H₂ [99].

7.2. Prevention of Cognitive Decline

Chronic physical restraint stress on mice enhanced levels of oxidative stress in the brain, and impaired learning and memory [100, 101]. Consumption of hydrogen water *ad libitum* suppressed the increase in oxidative stress, and prevented cognitive impairment. Neural proliferation in the dentate gyrus of the hippocampus was suppressed by restraint stress [101]. The consumption of hydrogen water ameliorated the reduced proliferation; however, a mechanistic link between H₂-dependent changes in neurogenesis and cognitive impairments remains unclear. Thus, continuous consumption of hydrogen water reduced oxidative stress in the brain and prevented the stress-induced decline in learning and memory [98].

7.3. Preventive and Therapeutic Affects on Parkinson Disease Model

In Parkinson's disease, mitochondrial dysfunction and the associated oxidative stress are major causes of dopaminergic cell loss in the substantia nigra [102]. H₂ in drinking water was given before or after stereotactic surgery for 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. Hydrogen water prevented both the development and progression of nigrostriatal degeneration. Hydrogen water likely retards the development and progression of Parkinson's disease [103].

Drinking hydrogen water suppressed dopaminergic neuronal loss in another Parkinson's disease model induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [104].

7.4. Prevention of Atherosclerosis Model

Oxidative stress is involved in atherosclerosis [105, 106]; however most clinical trials of dietary antioxidants failed to show marked success in preventing atherosclerotic diseases [8, 107, 108]. Drinking hydrogen water *ad libitum* decreased the aorta oxidative stress level and prevented arteriosclerosis in an apolipoprotein E knockout mouse [109]. Thus, consumption of hydrogen water has potential to prevent arteriosclerosis more effectively than other antioxidants [110].

7.5. Improvement of Metabolic Syndrome

Increased oxidative stress in obesity affects metabolic syndrome [111]. Long-term drinking of hydrogen water significantly controlled fat and body weights, despite no change in the consumption of food and water. Moreover, drinking hydrogen water decreased levels of plasma glucose, insulin and triglyceride, the effect of which on hyperglycemia was similar to diet restriction [112]. A mechanistic study revealed that the gene expression of the hepatic hormone, fibroblast growth factor 21 (FGF21) was enhanced, which should function to enhance fatty acid and glucose expenditure. Indeed, drinking hydrogen water stimulated energy metabolism, as measured by O₂ consumption and CO₂ expiration. These results suggest the potential benefit of H₂ in improving obesity, diabetes and metabolic syndrome [112].

7.6. Prevention of Adverse Effects by an Anti-tumor Drug

Cisplatin is a widely used anti-cancer drug in the treatment of a wide range of tumors; however, its application is limited by causing nephrotoxicity, which may be mediated by oxidative stress [113]. Inhalation of hydrogen gas (1% H₂ in air) or drinking hydrogen water improved mortality and body-weight loss caused by cisplatin, and alleviated nephrotoxicity. Consumption of hydrogen water improved metamorphosis accompanying decreased apoptosis in the kidney. Despite its protective effects against cisplatin-induced

toxicity, H₂ did not impair the anti-tumor activity of cisplatin against cancer cell lines *in vitro* and in tumor-bearing mice *in vivo*. Thus, H₂, whether hydrogen gas or hydrogen water, could improve the quality of life of patients during chemotherapy [99]. This finding was confirmed by another group [114].

7.7. Anti-allergic Reactions

It was demonstrated using a mouse model that drinking hydrogen water could attenuate an immediate-type allergic reaction by suppressing the phosphorylation of FcεRI-associated Lyn and its downstream signaling molecules, which subsequently inhibited NADPH oxidase activity and reduced the generation of hydrogen peroxide [115]. These findings imply that the beneficial effects of H₂ are not only imparted by its radical scavenging activity, but also by modulating a specific signaling pathway.

7.8. Effects on Transplantation

ROS contributes to the development of interstitial fibrosis and tubular atrophy seen in chronic allograft nephropathy. Nakao's group tested the effect of treatment with hydrogen water in a model of kidney transplantation, in which allografts from Lewis rats were orthotopically transplanted into Brown Norway recipients that had undergone bilateral nephrectomy. Drinking hydrogen water improved allograft function, slowed the progression of chronic allograft nephropathy, reduced oxidant injury and inflammatory mediator production, and improved overall survival. Inflammatory signaling pathways, such as mitogen-activated protein kinases, were less activated in renal allografts from hydrogen water-treated rats as compared with normal water-treated rats. Thus, oral hydrogen water is an effective antioxidant and anti-inflammatory agent that reduced chronic allograft nephropathy, improving the survival of rat renal allografts [116].

7.9. Others

It has been shown that drinking hydrogen water prevents superoxide formation in brain slices of vitamin C-depleted SMP30/GNL knockout mice [117], that H₂ in drinking water attenuates noise-induced hearing loss in guinea pigs [118], that drinking hydrogen water ameliorated cognitive impairment in senescence-accelerated mice [119], and that H₂ exhibited potential cardioprotective effects in irradiated mice [120].

8. METHODS OF INGEST HYDROGEN III: INJECTION OF HYDROGEN SALINE

8.1. Advantage of injection

Even though oral administration is safe and convenient, H₂ in water tends to escape over time and some H₂ is lost in the stomach or intestine, making it difficult to control the concentration of H₂ administered. Administration of H₂ via an injectable hydrogen saline (H₂-dissolved saline) vehicle may allow the delivery of more accurate concentrations of H₂ [121].

8.2. Effects of Hydrogen Saline on Various Disease Models

Sun's group administered H₂-saturated saline by peritoneal injection to various model animals with great success. Thus, hydrogen saline has potential in actual clinical treatment. For example, injection of hydrogen saline showed neuroprotective effects in a neonatal hypoxia-ischemia rat model [121]. Moreover, H₂ saline was applied to an Alzheimer's disease model mouse, which was generated by intracerebroventricular injection of the Aβ1-42 peptide. H₂ treatment decreased the level of oxidative stress and inflammation markers and prevented memory dysfunction and motor dysfunction, respectively [122].

They and other groups have demonstrated effects on many disease models, as published in the following reports [123-130].

9. METHODS OF INGEST HYDROGEN IV: DIRECT ABSORPTION OF HYDROGEN

9.1. Improvement of Glaucoma Model

Alternatively, H₂-loaded eye drops were prepared by dissolving H₂ in saline and directly administering to the ocular surface [131, 132].

In acute glaucoma of the eyes, transient elevation of intraocular pressure causes significant reductions in the thickness of the retina by ischemia-reperfusion injury mediated through the generation of reactive oxygen species [133]. The direct application of eye drops containing H₂ ameliorated ischemia-reperfusion injury of the retina in a rat model. When H₂ eye drops were continuously administered, the H₂ concentration increased in the vitreous body and the •OH level decreased during retinal ischemia-reperfusion. H₂ eye drops reduced the number of apoptotic and oxidative stress marker-positive cells 1 day after ischemia-reperfusion injury, and reduced retinal thinning with accompanying activation of Müller glia, astrocytes and microglia at 7 days after ischemia-reperfusion injury, improving the recovery of inner retinal layer thickness to >70%.

Moreover, we devised eye drops with dissolved H₂ to directly administer H₂ to the retina, and monitored the time course of changes in H₂ levels using a needle-shaped hydrogen sensor electrode inserted through the sclera to the vitreous body in rats. H₂ was able to reach the vitreous body by administering H₂ saturated in normal saline. When H₂ eye drops were administered continuously, approximately 70% H₂ was detected on the ocular surface. Two minutes after the start of administration, H₂ concentration in the vitreous body started to increase and reached a maximum level after 15 min. At that time, H₂ concentration was approximately 20% of H₂ in the eye-drops. The maximum concentration of H₂ in the vitreous body reached approximately one third of the value observed on the ocular surface [131].

9.2. Hydrogen Bath

H₂ easily penetrates the skin and distributes throughout the body via blood flow. Thus, taking a warm water bath with dissolved H₂ is a method of incorporating H₂ into the body in daily life, especially in Japan. It takes only 10 minutes to distribute throughout the whole body, as judged by measuring hydrogen gas in expiration (unpublished results).

10. METHODS OF INGEST HYDROGEN V: INCREASE IN INTESTINAL HYDROGEN

10.1. Production of Hydrogen in Intestinal Bacteria

Other medical gases, CO, NO and H₂S, are generated by endogenous enzymatic systems. Pharmaceutical development has taken advantage of these systems to design exogenous molecules to simulate those generated endogenously; however, mammals lack their own enzyme to produce H₂ [70].

Instead of endogenous enzymatic systems, the spontaneous production of hydrogen gas in the human body occurs via the fermentation of undigested carbohydrates by resident enterobacterial flora [134]. H₂ is transferred to the portal circulation and excreted through the breath in significant amounts [135]. For this reason, measurement of H₂ levels in expired air is used to detect carbohydrate malabsorption [76]; however, there have been few studies on the physiological function of gastrointestinal tract-derived hydrogen gas as an antioxidant.

10.2. Are α -glucosidase Inhibitors an Indirect Antioxidant?

α -Glucosidase inhibitors are pharmacological agents that specifically reduce postprandial hyperglycemia through retardation of disaccharide digestion, thereby reducing glucose absorption. A large scale epidemiologic trial has demonstrated that the treatment of patients with impaired glucose tolerance with an α -glucosidase

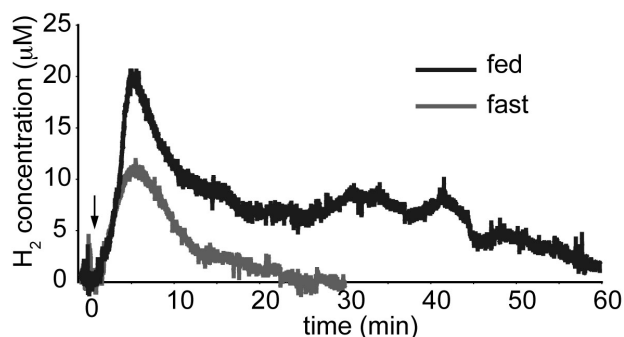
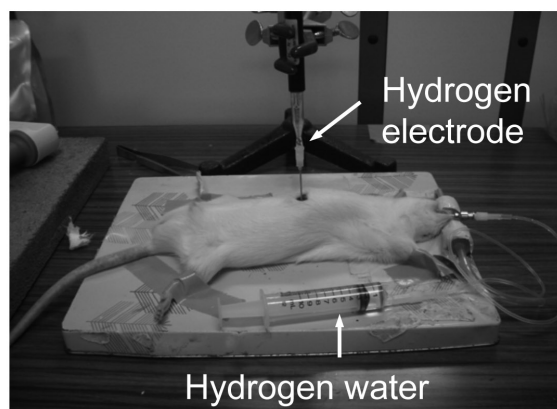


Fig. (2). Measurement of the accumulation of H₂ in rat liver.

The concentration of H₂ in the liver was monitored using a needle-type hydrogen sensor inserted into fed- or overnight fasted-rat liver. Rat received hydrogen water (0.8 mM H₂ in water) orally by stomach gavage at 15 ml/kg. Arrow indicates the time point when rat was administered hydrogen water.

inhibitor was associated with a 25% reduction in the risk of progression to diabetes, a 34% reduction in the risk of developing de novo hypertension, and a 49% risk reduction of cardiovascular events [136]. Furthermore, meta-analysis of seven long-term studies suggested that acarbose reduced the risk of myocardial infarction for patients with type 2 diabetes [137]. Such risk reduction for coronary heart disease events in patients with type 2 diabetes was not observed by improved glycemic control achieved by intensified treatment with insulin and glibenclamide [138]. Actually, acarbose, which is an α -glucosidase inhibitor, markedly increased H₂ production in volunteers. Thus, we propose that H₂ produced by intestinal bacteria acts as a unique antioxidant and prevents cardiovascular events [139].

10.3. Anti-inflammation Effects by Intestinal Bacteria via Hydrogen

Escherichia coli can produce a considerable amount of H₂ by catalyzing with hydrogenase. Kawai *et al.* examined whether H₂ released from intestinally colonized bacteria could affect concanavalin A-induced mouse hepatitis. Reconstitution of intestinal flora with H₂-producing *E. coli*, but not hydrogenase-deficient mutant *E. coli*, down-regulated concanavalin A-induced liver inflammation. These results indicate that H₂ released from intestinal bacteria can suppress inflammation [140]. H₂ also mediates the suppression of colon inflammation induced by dextran sodium sulfate [141].

10.4. Others

Dietary turmeric induced H₂ production from the intestinal bacteria [142], and lactulose was shown to be an indirect antioxidant ameliorating inflammatory bowel disease [143].

11. CLINICAL TESTS

Several groups have started clinical examinations. Clinical tests have revealed that drinking hydrogen water reduced oxidative stress markers in patients with type 2 diabetes [144] or subjects with potential metabolic syndrome [145] and influenced glucose [144] and cholesterol metabolism [145].

Hemodialysis using dialysis solution with H₂ significantly decreased the levels of plasma monocyte chemoattractant protein 1 and myeloperoxidase [146].

12. REGULATION OF GENE EXPRESSIONS AND PROTEIN PHOSPHORYLATIONS

It has been reported that H₂ acts as an anti-inflammatory and anti-allergic regulator by inducing inflammatory cytokines and inhibiting phosphorylating signal factors, respectively; however, the transcriptional factors and kinases involved in the effects afforded by H₂ have not been identified.

H₂ decreased the expressions of pro-inflammatory factors, including TNF- α , IL-6, IL-1 β , CCL2 and IL-10, TNF- γ , IL-12, ICAM-1 [85], HMGB-1 [147], NF- κ B [148], PGE2, and PGE2 [54].

Moreover, H₂ up- or down-regulated the factors involved in apoptosis toward the inhibition of apoptosis: H₂ suppressed the

expressions of pro-apoptotic factors, including caspase 3 [92, 149], and caspase 12 [92], caspase 8 [86] and BAX [86]. Conversely H₂ stimulated the expressions of the anti-apoptotic factors of Bcl-2 and Bcl-xL [86].

H₂ is involved in the regulation of various factors; up-regulation of PCNA, bFGF, HGF, IFN- γ , and down-regulation of i-NOS [87] and VEGF [54].

As a signal transduction contributor, H₂ inhibited the phosphorylations of some signal proteins, including MEK, p38, ERK, JNK [116] and Lyn, Syk, PLC γ 1, γ 2, Akt, ERK1/2, JNK, p38, cPLA2, ASK1, I κ B α [115].

Heme oxygenase-1 (HO-1), a microsomal enzyme degrading heme to carbon monoxide, free iron, and biliverdin, participates in the cell defense against oxidative stress and has been speculated to be a new therapeutic target [150]. Notably, H₂ modulates HO-1 expression, which is commonly up-regulated by these medical gases [48, 151]. Additionally, H₂ up-regulated the expression of FGF21, which is a regulator of energy metabolism [112].

As essential questions, it remains unknown how H₂ regulates gene expressions and phosphorylations, and whether the above regulations of transcription and phosphorylation are the cause or consequence of the effects of H₂. The primary molecular target of H₂ remains unknown.

Table 1. Diseases and Physiological States for Which Hydrogen Effects are Reported as Classified by Target Organs [152].

Disease/Physiology	Species	Source of H ₂	Reference
Brain			
Cerebral infarction	rodent	gas	[12]
Superoxide in brain	rodent	water	[117]
Neonatal brain hypoxia	rodent	gas	[92]
	rodent	saline	[131]
	pig	gas	[94]
Restraint-induced dementia	rodent	water	[98]
Alzheimer's disease	rodent	saline	[122]
Senile dementia	rodent	water	[119]
Parkinson's disease	rodent	water	[103, 104]
Hemorrhagic cerebral infarction	rodent	gas	[93]
Traumatic brain injury	rodent	gas	[95]
Spinal cord			
Spinal cord injury	rodent	saline	[130]
Eye			
Glaucoma	rodent	eye drops	[131]
Corneal alkali burn	rodent	eye drops	[132]
Ear			
Hearing disturbance	rodent	medium	[50]
	rodent	gas	[97]
	rodent	water	[118]

(Table 1) Contd....

Disease/Physiology	Species	Source of H ₂	Reference
Lung			
Oxygen-induced lung injury	rodent	saline	[128, 129]
Lung transplantation	rodent	gas	[86]
Heart			
Myocardial infarction	rodent	gas	[58]
	rodent	saline	[149]
Heart transplantation	rodent	gas	[87]
Irradiation-induced heart injury	rodent	water	[120]
Liver			
Hepatic ischemia	rodent	gas	[84]
Hepatitis	rodent	bacteria	[140]
Obstructive jaundice	rodent	saline	[124]
Kidney			
Cisplatin nephropathy	rodent	gas, water	[99]
	rodent	water	[114]
Hemodialysis	human	dialysis	[146]
Kidney transplantation	rodent	water	[116]
Pancreas			
Acute pancreatitis	rodent	saline	[148]
Intestine			
Intestinal graft	rodent	gas	[85]
	rodent	saline	[125, 130]
Ulcerative colitis	rodent	gas	[141]
Blood vessel			
Atherosclerosis	rodent	water	[110]
Metabolism			
Diabetes mellitus type 2	human	water	[144]
Metabolic syndrome	human	water	[145]
Obesity/Diabetes	rodent	water	[112]
Inflammation and allergy			
Allergy type I	rodent	water	[115]
Sepsis	rodent	gas	[90]
Zymosan-induced inflammation	rodent	gas	[91]
Others			
Multipotent stromal cells	cells	medium	[54]
Radiation injury	cells, rodent	medium	[52]

13. CLOSING REMARKS: ISSUES TO BE DISSOLVED IN THE FUTURE

In our first report published in 2007, we indicated that H₂ reacted with strong reactive oxygen/nitrogen species, including •OH and ONOO⁻ in cell-free reactions. Cells cultured in H₂-rich medium were protected against oxidative stress by the •OH-scavenging activity of H₂, depending upon the decrease of •OH [12]; however, recent evidence shows that the scavenging property is not the only explanation for the potent beneficial effects of H₂. When model animals and human subjects consumed H₂ by drinking water with dissolved H₂, even a very small amount of H₂ was extensively effective. It may be difficult to explain that direct reduction of •OH by a very small amount of H₂ reveals all the functions of H₂, because the saturated level of H₂ is only 0.8 mM and the dwelling time of •OH is very short in the body.

We have recently shown that H₂ can be accumulated with hepatic glycogen; this finding indicates the possible accumulation of H₂ in a specific region; however, it is unlikely that the amount of H₂ is sufficient to exhibit all of its functions [112]. Additionally, drinking 0.04 or 0.08 mM H₂ was shown to be effective [104, 112]. The amount of administered H₂ seems to be, in many cases, independent of the magnitude of effects. Intestinal bacteria produce more than 1 liter of hydrogen gas per day, whereas the amount of H₂ originating from drinking hydrogen water is less than 50 ml. Nevertheless, additional H₂ in drinking hydrogen water is unambiguously effective.

Many additional issues of hydrogen therapy including the molecular mechanisms underlying the marked effects of a very small amount of H₂ remain elusive. The primary molecular target of H₂ remains unknown. Although H₂ regulates various gene expressions and protein-phosphorylations, it remains unclear whether such regulations are the cause or consequence of the effects against oxidative stress. One of the open questions is how H₂ involves the cross-talk among anti-oxidation, anti-inflammation and anti-allergy. Thus, it should not be fair to classify the roles of H₂ by outward effects at this stage.

Finally, the author summarizes the reports showing the effects of H₂ by the classification of target organs (Table 1) [152].

DISCLOSURE

The author declares no conflicts of interest.

REFERENCES

- [1] Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005; 39: 359-407.
- [2] Ferrari R, Ceconi C, Cargnoni A, Pasini E, Visioli O. The occurrence of oxidative stress during reperfusion in experimental animals and men. *Cardiovasc Drugs Ther* 1991; 5 Suppl 2: 277-87.
- [3] Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? *Nat Med* 2004; 10 Suppl: S18-25.
- [4] Ohta S, Ohsawa I. Dysfunction of mitochondria and oxidative stress in the pathogenesis of Alzheimer's disease: on defects in the cytochrome c oxidase complex and aldehyde detoxification. *J Alzheimers Dis* 2006; 9: 155-66.
- [5] Ohta S, Ohsawa I, Kamino K, Ando F, Shimokata H. Mitochondrial ALDH2 deficiency as an oxidative stress. *Ann N Y Acad Sci* 2004; 1011: 36-44.
- [6] Chang JC, Kou SJ, Lin WT, Liu CS. Regulatory role of mitochondria in oxidative stress and atherosclerosis. *World J Cardiol* 2010; 2: 150-9.
- [7] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; 408: 239-47.
- [8] Steinhubl SR. Why have antioxidants failed in clinical trials? *Am J Cardiol* 2008; 101: 14D-9D.
- [9] Hercberg S, Kesse-Guyot E, Druetne-Pecollo N, *et al.* Incidence of cancers, ischemic cardiovascular diseases and mortality during 5-year follow-up after stopping antioxidant vitamins and minerals supplements: a postintervention follow-up in the SU.VI.MAX Study. *Int J Cancer* 2010; 127: 1875-81.
- [10] Brambilla D, Mancuso C, Scuderi MR, *et al.* The role of antioxidant supplement in immune system, neoplastic, and neurodegenerative disorders: a point of view for an assessment of the risk/benefit profile. *Nutr J* 2008; 7: 29.
- [11] Hackam DG. Review: antioxidant supplements for primary and secondary prevention do not decrease mortality. *ACP J Club* 2007; 147: 4.
- [12] 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.
- [13] Salganik RI. The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population. *J. Am. Coll. Nutr* 2001; 20: 464S-72S; discussion 73S-75S.
- [14] Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell. Physiol. Biochem* 2001; 11: 173-86.
- [15] Liu H, Colavitti R, Rovira, II, Finkel T. Redox-dependent transcriptional regulation. *Circ. Res* 2005; 97: 967-74.
- [16] Harma MI, Harma M, Erel O. Measuring plasma oxidative stress biomarkers in sport medicine. *Eur J Appl Physiol* 2006; 97: 505; author reply 6-8.
- [17] Tanriverdi H, Evrengul H, Kuru O, *et al.* Cigarette smoking induced oxidative stress may impair endothelial function and coronary blood flow in angiographically normal coronary arteries. *Circ J* 2006; 70: 593-9.
- [18] Grassi D, Desideri G, Ferri L, Aggio A, Tiberti S, Ferri C. Oxidative stress and endothelial dysfunction: say no to cigarette smoking! *Curr Pharm Des* 2010; 16: 2539-50.
- [19] Agarwal R. Smoking, oxidative stress and inflammation: impact on resting energy expenditure in diabetic nephropathy. *BMC Nephrol* 2005; 6: 13.
- [20] Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 2003; 552: 335-44.
- [21] Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 2006; 443: 787-95.
- [22] Halliwell B, Gutteridge JM. Biologically relevant metal ion-dependent hydroxyl radical generation. An update. *FEBS Lett* 1992; 307: 108-12.
- [23] Peled-Kamar M, Lotem J, Wirguin I, Weiner L, Hermalin A, Groner Y. Oxidative stress mediates impairment of muscle function in transgenic mice with elevated level of wild-type Cu/Zn superoxide dismutase. *Proc Natl Acad Sci U S A* 1997; 94: 3883-7.
- [24] Chan PH, Epstein CJ, Li Y, *et al.* Transgenic mice and knockout mutants in the study of oxidative stress in brain injury. *J Neurotrauma* 1995; 12: 815-24.
- [25] Mitsui A, Hamuro J, Nakamura H, *et al.* Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. *Antioxid Redox Signal* 2002; 4: 693-6.
- [26] Stefanova N, Reindl M, Neumann M, *et al.* Oxidative stress in transgenic mice with oligodendroglial alpha-synuclein overexpression replicates the characteristic neuropathology of multiple system atrophy. *Am J Pathol* 2005; 166: 869-76.
- [27] Schriener SE, Linford NJ, Martin GM, *et al.* Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 2005; 308: 1909-11.
- [28] Stockl P, Zankl C, Hutter E, *et al.* Partial uncoupling of oxidative phosphorylation induces premature senescence in human fibroblasts and yeast mother cells. *Free Radic Biol Med* 2007; 43: 947-58.
- [29] Jia JJ, Zhang X, Ge CR, Jois M. The polymorphisms of UCP2 and UCP3 genes associated with fat metabolism, obesity and diabetes. *Obes Rev* 2009; 10: 519-26.
- [30] Jia JJ, Tian YB, Cao ZH, *et al.* The polymorphisms of UCP1 genes associated with fat metabolism, obesity and diabetes. *Mol. Biol. Rep* 2010; 37: 1513-22.
- [31] Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ. Res* 2010; 107: 1058-70.
- [32] Ohsawa I, Nishimaki K, Murakami Y, Suzuki Y, Ishikawa M, Ohta S. Age-dependent neurodegeneration accompanying memory loss in transgenic mice defective in mitochondrial aldehyde dehydrogenase 2 activity. *J Neurosci* 2008; 28: 6239-49.
- [33] Endo J, Sano M, Katayama T, Hishiki T, *et al.* Metabolic remodeling induced by mitochondrial aldehyde stress stimulates

- tolerance to oxidative stress in the heart. *Circ Res* 2009; 105: 1118-27.
- [34] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* 2010; 49: 1603-16.
- [35] Vaziri ND, Rodriguez-Iturbe B. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. *Nat Clin Pract Nephrol* 2006; 2: 582-93.
- [36] Bolli R, Jeroudi MO, Patel BS, *et al.* Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury. *Circ Res* 1989; 65: 607-22.
- [37] Zweier JL. Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. *J Biol Chem* 1988; 263: 1353-7.
- [38] Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitron. *J Clin Invest* 1988; 82: 476-85.
- [39] Vanden Hoek T, Becker LB, Shao ZH, Li CQ, Schumacker PT. Preconditioning in cardiomyocytes protects by attenuating oxidant stress at reperfusion. *Circ Res* 2000; 86: 541-8.
- [40] Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch. Biochem. Biophys* 1986; 246: 501-14.
- [41] Halestrap AP, Clarke SJ, Khaliulin I. The role of mitochondria in protection of the heart by preconditioning. *Biochim Biophys Acta* 2007; 1767: 1007-31.
- [42] Flaherty JT, Pitt B, Gruber JW, Heuser RR, Rothbaum DA, Burwell LR, George BS, Kereiakes DJ, Deitchman D, Gustafson N, *et al.* Recombinant human superoxide dismutase (h-SOD) fails to improve recovery of ventricular function in patients undergoing coronary angioplasty for acute myocardial infarction. *Circulation* 1994; 89: 1982-91.
- [43] Richard VJ, Murry CE, Jennings RB, Reimer KA. Therapy to reduce free radicals during early reperfusion does not limit the size of myocardial infarcts caused by 90 minutes of ischemia in dogs. *Circulation* 1988; 78: 473-80.
- [44] Ristow M, Zarse K. How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp Gerontol* 2010; 45: 410-8.
- [45] Ristow M, Zarse K, Oberbach A, *et al.* Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* 2009; 106: 8665-70.
- [46] Penna C, Rastaldo R, Mancardi D, *et al.* Post-conditioning induced cardioprotection requires signaling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K⁺ channel and protein kinase C activation. *Basic Res Cardiol* 2006; 101: 180-9.
- [47] Downey JM, Cohen MV. A really radical observation--a comment on Penna *et al.* in *Basic Res Cardiol* (2006) 101:180-189. *Basic Res Cardiol* 2006; 101: 190-1.
- [48] Huang CS, Kawamura T, Toyoda Y, Nakao A. Recent advances in hydrogen research as a therapeutic medical gas. *Free Radic Res* 2010; 44: 971-82.
- [49] Setsukinai K, Urano Y, Kakinuma K, Majima HJ, Nagano T. Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. *J Biol Chem* 2003; 278: 3170-5.
- [50] Kikkawa YS, Nakagawa T, Horie RT, Ito J. Hydrogen protects auditory hair cells from free radicals. *Neuroreport* 2009; 20: 689-94.
- [51] Taura A, Kikkawa YS, Nakagawa T, Ito J. Hydrogen protects vestibular hair cells from free radicals. *Acta Otolaryngol Suppl* 2010; 95:100.
- [52] Qian L, Cao F, Cui J, *et al.* Radioprotective effect of hydrogen in cultured cells and mice. *Free Radic Res* 2010; 44: 275-82.
- [53] Schoenfeld MP, Ansari RR, Zakrajsek JF, *et al.* Hydrogen therapy may reduce the risks related to radiation-induced oxidative stress in space flight. *Med Hypotheses* 2011; 76: 117-8.
- [54] Kawasaki H, Guan J, Tamama K. Hydrogen gas treatment prolongs replicative lifespan of bone marrow multipotential stromal cells *in vitro* while preserving differentiation and paracrine potentials. *Biochem Biophys Res Commun* 2010; 397: 608-13.
- [55] Murphy MP, Smith RA. Drug delivery to mitochondria: the key to mitochondrial medicine. *Adv Drug Deliv Rev* 2000; 41: 235-50.
- [56] Murphy MP. Selective targeting of bioactive compounds to mitochondria. *Trends Biotechnol* 1997; 15: 326-30.
- [57] Smith RA, Murphy MP. Mitochondria-targeted antioxidants as therapies. *Discov Med* 2011; 11: 106-14.
- [58] Hayashida K, Sano M, Ohsawa I, *et al.* Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury. *Biochem Biophys Res Commun* 2008; 373: 30-5.
- [59] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007; 297: 842-57.
- [60] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev* 2008; CD007176.
- [61] Gray SL, Anderson ML, Crane PK, *et al.* Antioxidant vitamin supplement use and risk of dementia or Alzheimer's disease in older adults. *J Am Geriatr Soc* 2008; 56: 291-5.
- [62] Walker C. Antioxidant supplements do not improve mortality and may cause harm. *Am Fam Physician* 2008; 78: 1079-80.
- [63] Bjelakovic G, Gluud C. Surviving antioxidant supplements. *J. Natl. Cancer Inst* 2007; 99: 742-3.
- [64] Miller ER, 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med* 2005; 142: 37-46.
- [65] Mandal CC, Ganapathy S, Gorin Y, *et al.* Reactive oxygen species derived from Nox4 mediate BMP2 gene transcription and osteoblast differentiation. *Biochem J* 2010; 433: 393-402.
- [66] Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A* 1998; 95: 11715-20.
- [67] Carriere A, Carmona MC, Fernandez Y, *et al.* Mitochondrial reactive oxygen species control the transcription factor CHOP-10/GADD153 and adipocyte differentiation: a mechanism for hypoxia-dependent effect. *J Biol Chem* 2004; 279: 40462-9.
- [68] Winterbourn CC. Biological reactivity and biomarkers of the neutrophil oxidant, hypochlorous acid. *Toxicology* 2002; 181-182: 223-7.
- [69] Murad F. Discovery of some of the biological effects of nitric oxide and its role in cell signaling. *Biosci. Rep* 2004; 24: 452-74.
- [70] Kajimura M, Fukuda R, Bateman RM, Yamamoto T, Suematsu M. Interactions of multiple gas-transducing systems: hallmarks and uncertainties of CO, NO, and H₂S gas biology. *Antioxid Redox Signal* 2010; 13: 157-92.
- [71] Motterlini R, Otterbein LE. The therapeutic potential of carbon monoxide. *Nat Rev Drug Discov* 2010; 9: 728-43.
- [72] Kimura H. Hydrogen sulfide: from brain to gut. *Antioxid Redox Signal* 2010; 12: 1111-23.
- [73] Szabo C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 2007; 6: 917-35.
- [74] Elrod JW, Calvert JW, Morrison J, *et al.* Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci U S A* 2007; 104: 15560-5.
- [75] Foresti R, Bani-Hani MG, Motterlini R. Use of carbon monoxide as a therapeutic agent: promises and challenges. *Intensive Care Med* 2008.
- [76] Kobayashi A, Ishikawa K, Matsumoto H, Kimura S, Kamiyama Y, Maruyama Y. Synergistic antioxidant and vasodilatory action of carbon monoxide in angiotensin II - induced cardiac hypertrophy. *Hypertension* 2007; 50: 1040-8.
- [77] Bloch KD, Ichinose F, Roberts JD, Jr., Zapol WM. Inhaled NO as a therapeutic agent. *Cardiovasc Res* 2007; 75: 339-48.
- [78] Abbraini JH, Gardette-Chauffour MC, Martinez E, Rostain JC, Lemaire C. Psychophysiological reactions in humans during an open sea dive to 500 m with a hydrogen-helium-oxygen mixture. *J Appl Physiol* 1994; 76: 1113-8.
- [79] Lillo RS, Parker EC, Porter WR. Decompression comparison of helium and hydrogen in rats. *J Appl Physiol* 1997; 82: 892-901.
- [80] Lillo RS, Parker EC. Mixed-gas model for predicting decompression sickness in rats. *J Appl Physiol* 2000; 89: 2107-16.
- [81] Fontanari P, Badier M, Guillot C, *et al.* Changes in maximal performance of inspiratory and skeletal muscles during and after

- the 7.1-MPa Hydra 10 record human dive. *Eur J Appl Physiol* 2000; 81: 325-8.
- [82] Peters O, Back T, Lindauer U, *et al.* Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab* 1998; 18: 196-205.
- [83] Jaeschke H, Smith CV, Mitchell JR. Reactive oxygen species during ischemia-reflow injury in isolated perfused rat liver. *J. Clin. Invest* 1988; 81: 1240-6.
- [84] 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.
- [85] Buchholz BM, Kaczorowski DJ, Sugimoto R, *et al.* Hydrogen inhalation ameliorates oxidative stress in transplantation induced intestinal graft injury. *Am J Transplant* 2008; 8: 2015-24.
- [86] Kawamura T, Huang CS, Tochigi N, *et al.* Inhaled hydrogen gas therapy for prevention of lung transplant-induced ischemia/reperfusion injury in rats. *Transplantation* 2010; 90: 1344-51.
- [87] Nakao A, Kaczorowski DJ, Wang Y, *et al.* Amelioration of rat cardiac cold ischemia/reperfusion injury with inhaled hydrogen or carbon monoxide, or both. *J Heart Lung Transplant* 2010; 29: 544-53.
- [88] Huang CS, Kawamura T, Lee S, *et al.* Hydrogen inhalation ameliorates ventilator-induced lung injury. *Crit Care* 2010; 14: R234.
- [89] Victor VM, Espulgues JV, Hernandez-Mijares A, Rocha M. Oxidative stress and mitochondrial dysfunction in sepsis: a potential therapy with mitochondria-targeted antioxidants. *Infect Disord Drug Targets* 2009; 9: 376-89.
- [90] Xie KL, Yu YH, Pei YP, *et al.* Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. *Shock* 2010; 34: 90-7.
- [91] Xie K, Yu Y, Zhang Z, *et al.* Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model. *Shock* 2010; 34: 495-501.
- [92] Cai J, Kang Z, Liu WW, *et al.* Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model. *Neurosci Lett* 2008; 441: 167-72.
- [93] Chen CH, Manaenko A, Zhan Y, Liu WW, Ostrowki RP, Tang J, Zhang JH. Hydrogen gas reduced acute hyperglycemia-enhanced hemorrhagic transformation in a focal ischemia rat model. *Neuroscience* 2010; 169: 402-14.
- [94] Domoki F, Olah O, Zimmermann A, *et al.* Hydrogen is neuroprotective and preserves cerebrovascular reactivity in asphyxiated newborn pigs. *Pediatr Res* 2010; 68: 387-92.
- [95] Ji X, Liu W, Xie K, *et al.* Beneficial effects of hydrogen gas in a rat model of traumatic brain injury via reducing oxidative stress. *Brain Res* 2010; 1354: 196-205.
- [96] Huang Y, Xie K, Li J, *et al.* Beneficial effects of hydrogen gas against spinal cord ischemia-reperfusion injury in rabbits. *Brain Res* 2011; 1378: 125-36.
- [97] Taura A, Kikkawa YS, Nakagawa T, Ito J. Hydrogen protects vestibular hair cells from free radicals. *Acta Otolaryngol. (Stockh)* 2010; 130: 95-100.
- [98] 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.
- [99] Nakashima-Kamimura N, Mori T, Ohsawa I, Asoh S, Ohta S. Molecular hydrogen alleviates nephrotoxicity induced by an anti-cancer drug cisplatin without compromising anti-tumor activity in mice. *Cancer Chemother Pharmacol* 2009; 64: 753-61.
- [100] Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. *FASEB J* 1996; 10: 1532-8.
- [101] Abrous DN, Koehl M, Le Moal M. Adult neurogenesis: from precursors to network and physiology. *Physiol Rev* 2005; 85: 523-69.
- [102] Schapira AH. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol* 2008; 7: 97-109.
- [103] 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.
- [104] 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.
- [105] Victor VM, Apostolova N, Herance R, Hernandez-Mijares A, Rocha M. Oxidative stress and mitochondrial dysfunction in atherosclerosis: mitochondria-targeted antioxidants as potential therapy. *Curr Med Chem* 2009; 16: 4654-67.
- [106] Stocker R, Keaney JF, Jr. Role of oxidative modifications in atherosclerosis. *Physiol. Rev* 2004; 84: 1381-478.
- [107] Upston JM, Kritharides L, Stocker R. The role of vitamin E in atherosclerosis. *Prog. Lipid Res* 2003; 42: 405-22.
- [108] Hodis HN, Mack WJ, LaBree L, *et al.* Alpha-tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). *Circulation* 2002; 106: 1453-9.
- [109] Kolovou G, Anagnostopoulou K, Mikhailidis DP, Cokkinos DV. Apolipoprotein E knockout models. *Curr Pharm Des* 2008; 14: 338-51.
- [110] Ohsawa I, Nishimaki K, Yamagata K, Ishikawa M, Ohta S. Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice. *Biochem Biophys Res Commun* 2008; 377: 1195-8.
- [111] Furukawa S, Fujita T, Shimabukuro M, *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; 114: 1752-61.
- [112] 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 (Silver Spring)* 2011. in press.
- [113] Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. *Am J Med Sci* 2007; 334: 115-24.
- [114] Kitamura A, Kobayashi S, Matsushita T, Fujinawa H, Murase K. Experimental verification of protective effect of hydrogen-rich water against cisplatin-induced nephrotoxicity in rats using dynamic contrast-enhanced CT. *Br. J. Radiol* 2010; 83: 509-14.
- [115] Itoh T, Fujita Y, Ito M, *et al.* Molecular hydrogen suppresses FcεpsilonRI-mediated signal transduction and prevents degranulation of mast cells. *Biochem Biophys Res Commun* 2009; 389: 651-6.
- [116] Cardinal JS, Zhan J, Wang Y, *et al.* Oral hydrogen water prevents chronic allograft nephropathy in rats. *Kidney Int* 2010; 77: 101-9.
- [117] Sato Y, Kajiyama S, Amano A, *et al.* Hydrogen-rich pure water prevents superoxide formation in brain slices of vitamin C-depleted SMP30/GNL knockout mice. *Biochem Biophys Res Commun* 2008; 375: 346-50.
- [118] Lin Y, Kashio A, Sakamoto T, Suzukawa K, Kakigi A, Yamasoba T. Hydrogen in drinking water attenuates noise-induced hearing loss in guinea pigs. *Neurosci Lett* 2011; 487: 12-6.
- [119] Gu Y, Huang CS, Inoue T, Yamashita T, Ishida T, Kang KM, Nakao A. Drinking Hydrogen Water Ameliorated Cognitive Impairment in Senescence-Accelerated Mice. *Journal of Clinical Biochemistry and Nutrition* 2010; 46: 269-76.
- [120] Qian LR, Cao F, Cui JG, *et al.* The Potential Cardioprotective Effects of Hydrogen in Irradiated Mice. *J. Radiat. Res. (Tokyo)* 2010; 51: 741-7.
- [121] Cai JM, Kang ZM, Liu K, *et al.* Neuroprotective effects of hydrogen saline in neonatal hypoxia-ischemia rat model. *Brain Res* 2009; 1256: 129-37.
- [122] 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.
- [123] Chen C, Chen Q, Mao Y, *et al.* Hydrogen-rich saline protects against spinal cord injury in rats. *Neurochem Res* 2010; 35: 1111-8.
- [124] 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.
- [125] 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.
- [126] Qian L, Cao F, Cui J, *et al.* The potential cardioprotective effects of hydrogen in irradiated mice. *J Radiat Res (Tokyo)* 2010; 51: 741-7.

- [127] Shingu C, Koga H, Hagiwara S, Matsumoto S, Goto K, Yokoi I, Noguchi T. Hydrogen-rich saline solution attenuates renal ischemia-reperfusion injury. *J Anesth* 2010; 24: 569-74.
- [128] Sun Q, Cai J, Liu S, Liu Y, Xu W, Tao H, Sun X. Hydrogen-rich saline provides protection against hyperoxic lung injury. *J Surg Res* 2011; 165: e43-9.
- [129] Zheng J, Liu K, Kang Z, *et al.* Saturated hydrogen saline protects the lung against oxygen toxicity. *Undersea Hyperb Med* 2010; 37: 185-92.
- [130] Zheng X, Mao Y, Cai J, *et al.* Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats. *Free Radic Res* 2009; 43: 478-84.
- [131] 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.
- [132] 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.
- [133] Nakabayashi M. Review of the ischemia hypothesis for ocular hypertension other than congenital glaucoma and closed-angle glaucoma. *Ophthalmologica* 2004; 218: 344-9.
- [134] Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* 1977; 41: 100-80.
- [135] Levitt MD. Production and excretion of hydrogen gas in man. *N. Engl. J. Med* 1969; 281: 122-7.
- [136] Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; 290: 486-94.
- [137] Hanefeld M, Cagatay M, Petrowitsch T, Neuser D, Petzinna D, Rupp M. Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. *Eur. Heart J* 2004; 25: 10-6.
- [138] Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; 352: 837-53.
- [139] Suzuki Y, Sano M, Hayashida K, Ohsawa I, Ohta S, Fukuda K. Are the effects of alpha-glucosidase inhibitors on cardiovascular events related to elevated levels of hydrogen gas in the gastrointestinal tract? *FEBS Lett* 2009; 583: 2157-9.
- [140] 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.
- [141] Kajiya M, Silva MJ, Sato K, Ouhara K, Kawai T. Hydrogen mediates suppression of colon inflammation induced by dextran sodium sulfate. *Biochem Biophys Res Commun* 2009; 386: 11-5.
- [142] Shimouchi A, Nose K, Takaoka M, Hayashi H, Kondo T. Effect of dietary turmeric on breath hydrogen. *Dig Dis Sci* 2009; 54: 1725-9.
- [143] Chen X, Zuo Q, Hai Y, Sun XJ. Lactulose: an indirect antioxidant ameliorating inflammatory bowel disease by increasing hydrogen production. *Med Hypotheses* 2011; 76: 325-7.
- [144] Kajiya M, 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.
- [145] 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.
- [146] 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.
- [147] Xie K, Yu Y, Pei Y, Hou L, Chen S, Xiong L, Wang G. Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. *Shock* 2010; 34: 90-7.
- [148] Chen H, Sun YP, Li Y, *et al.* Hydrogen-rich saline ameliorates the severity of l-arginine-induced acute pancreatitis in rats. *Biochem Biophys Res Commun* 2010; 393: 308-13.
- [149] Sun Q, Kang Z, Cai J, *et al.* Hydrogen-rich saline protects myocardium against ischemia/reperfusion injury in rats. *Exp Biol Med* (Maywood) 2009; 234: 1212-9.
- [150] Jazwa A, Cuadrado A. Targeting heme oxygenase-1 for neuroprotection and neuroinflammation in neurodegenerative diseases. *Curr Drug Targets* 2010; 11: 1517-31.
- [151] Park DJ, Agarwal A, George JF. Heme oxygenase-1 expression in murine dendritic cell subpopulations: effect on CD8+ dendritic cell differentiation *in vivo*. *Am J Pathol* 2010; 176: 2831-9.
- [152] Ohta S, Nakao A, Ohno K. The 2011 Medical Molecular Hydrogen Symposium: An Inaugural Symposium of the Journal Medical Gas Research. *Medical Gas Research* 2011; 1: 10.