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Molecular hydrogen as a new selective anti–oxidation therapeutic medical gas for clinical research

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ABSTRACT

Hydrogen (H_2) is a colorless, odorless gas that can act as a reducing agent under certain circumstances. Previously considered physiologically an inert and nonfunctional molecule in mammalian cells, H_2 largely went ignored until *Nature Medicine* revealed the antioxidant and cytoprotective effects of hydrogen gas in a focal stroke model. Reactive oxygen species (ROS) is generated inside the body throughout our daily lives as a by–product of the energy metabolism by oxidative phosphorylation, which can cause biofilm system damage and intracellular oxidative phosphorylation disorders. H_2 reacts with highly reactive oxidants such as hydroxyl radical (•OH) and peroxynitrite ($ONOO^-$) inside cells to improve ischemia reperfusion injury. In addition, hydrogen is a potent antiapoptotic and anti–inflammatory agent and can be used for potential medical applications in cells, tissues and organs. As a new antioxidant, hydrogen has the advantages of non–toxicity, easy diffusion and selective neutralization. This review makes a case for supporting hydrogen as a new antioxidant medicine for clinical applications. We also hope to provide a reference for the further study of hydrogen to preserve blood cells in transfusion medicine.

Keywords: hydrogen, reactive oxygen species, selective antioxidation

INTRODUCTION

Reactive oxygen species (ROS) is generated inside the body throughout our daily lives as a by–product of the energy metabolism by oxidative phosphorylation in every aerobic organism, including superoxide anion (O^{2-}), hydroxyl radical (•OH), hydroperoxide free radical (HOO•), peroxynitrite ($ONOO^-$) and so on. It is known to increase rapidly under certain types of *physical* exposure (such as ultraviolet or irradiation rays) or chemical exposure (such as aromatic hydrocarbons or pesticides). Usually, the body can limit damage caused by enzymes (such as superoxide dismutase). However, if ROS is produced excessively or the endogenous antioxidant capacity is diminished (exceeding the body´s compensatory capacity), an imbalance between *oxidation* and *antioxidation* will occur and leading to pathological damage, known as oxidative stress. The main molecules of oxidative stress are the hydroxyl radical and peroxynitrite which can damage the body by direct action or triggering a free radical chain reaction[1]. Evidence has established strong links between oxidative stress and a wide variety of pathologies, including apoptosis, aging process, chronic inflammatory processes as well as malignant diseases[2].

As a reducing agent, H_2 can scavenge deleterious ROS by selectively reacting with ROS (such as hydroxyl radical or peroxynitrite) *in vivo*, meaning that H_2 has preventive and therapeutic applications in alleviating oxidative stress related diseases. It´s three main advantages in antioxidation: Firstly, H_2 as a gas,

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has a very low molecular weight, causing a high bi–omembrane penetration and intracellular diffusion capability which enhances cell affinity[3]. Secondly, H_2 can selectively scavenge the deleterious hydroxyl radical or peroxynitrite while preserving other impor–tant ROS (such as H_2O_2, O_2^- or NO, etc.) for normal signaling regulation, therefore being superior to other antioxidants with a strong reductive activity (such as vitamin C) which ensures safe application[3]. Thirdly, H_2 has no cytotoxicity (even at high concentrations) which avoids the increased risk of mortality associated with other gases[4]. Owing to its selective antioxidation and lack of adverse effects, H_2 has promising potential for clinical therapeutic applications.

MOLECULAR MECHANISM

Reduction of hydroxyl radical

ROS serve a necessary function as signaling mol–ecules that critically modulate the activation of the immune system and thus participate in body defense. The cells contain natural radical scavengers, which as long as they are enough to neutralize radiolysis products, and the DNA may be protected. Conversely, when radiolysis products exceed the amount of scav–engers, they produce•OH, which directly affect the macromolecules in cells, resulting in DNA breakage, lipid peroxidation, protein denaturation and so on, after which radiation damage and cancer induction may occur[5]. In conclusion, •OH are highly toxic to the body, and can cause a free radical chain reaction which transmits signals through the cell membrane to produce lipid peroxides and oxidative stress products. In supporting research, Ning *et al*.[6] confirmed that H_2 can reduce the expression of ROS and oxidative stress products. While Ohta[7] demonstrated that H_2 reacts with strong oxidants such as hydroxyl radicals within cells. H_2 rapidly diffuses into tissues and cells, and it is mild enough neither to disturb metabolic redox reactions nor to affect the function of ROS in cellular signaling[7].

Reduction of peroxynitrite

The nitric oxide (NO) can readily react with O^{2-} to produce peroxynitrite, which participates as a media–tor in the regulation of vascular tone, neurotransmis–sion, and immunity, among other metabolic and cell signaling effects[8]. Although H_2 can´t scavenge per–oxynitrite, it is able to diminish the toxicity of perox–ynitrite[9]. This is in accordance with previous studies, which established that H_2 or hydrogen rich water can reduce the concentration of peroxynitrite in animal models[10–12].

Regulating endogenous signal pathway

Oxidative stress impacts multiple signaling path–ways, including the extracellular signal–regulated protein kinase (ERK)1/2, NF–κB, as well as nuclear factor–erythroid–related factor 2 (Nrf2) pathways. Along with selectively scavenging •OH, H_2 may al–leviate oxidative stress–induced injury by targeting these pathways[13]. Ke *et al*.[14] has shown that Nrf2 can inhibit the inflammatory response in liver trans–planted mice and inhibit hepatocellular necrosis/apoptosis through hypoxia–inducible factor(HIF)–1α factor. Kawamura[15] demonstrated that inhaled 2% H_2 can ameliorate hyperoxic lung injury through the induction of Nrf2–dependent genes, such as heme oxygenase(HO)–1. In conclusion, H_2 may play an antioxidant role by activating Nrf2 and raising target protein expression.

Indirect effects

The body has many antioxidant enzymes, includ–ing superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (glutathione peroxidase, GSH–Px). The study showed that H_2 can reduce oxidative stress damage by increasing endogenous antioxidant enzyme activity[16]. In summary, H_2 can not only di–rectly react to reduce oxidative stress damage, but also can indirectly exert antioxidant effects by inducing the endogenous antioxidant system.

METHODS OF INGESTING HYDROGEN

Inhalation of hydrogen

A direct method of obtaining H_2 therapeutically is through inhalation by using a ventilator circuit, face–mask, or nasal cannula. Patients typically inhale H_2 through a facemask, whereas in animal models, H_2 is commonly obtained through a ventilator that provides H_2 electrolyzed from water. Inhaled H_2 acts rapidly and may be used to treat acute oxidative stress[7]. Moreover, H_2 inhalation is a safe and effective method for patients with acute diseases[17].

Oral ingestion of hydrogen–rich water

H_2 can be dissolved in water up to 0.8 mmol/L (1.6 mg/L) under atmospheric pressure at room tempera–ture without changing pH. H_2–rich water can be made by 3 methods: firstly, by infusing H_2 into water un–der high pressure; secondly, by electrolyzing water; thirdly, though the reaction of magnesium metal or its hydride with water. However, H_2 must be stored cor–rectly as it easily penetrates glass or plastic containers in a short time, with aluminum containers being the

preferred choice for its storage[18,19]. Currently, H_2−rich water is distributed, and available for purchase.

Injection of hydrogen−rich saline

Although administering oral hydrogen−rich water is safe and convenient, controlling the concentration of H_2 obtained can be difficult, as it evaporates over time and can be lost before absorption in the gastrointestinal tract. Thus, hydrogen−rich saline injection may be used to control H_2 doses accurately[20,21]. Nagatani *et al*.[22] indicated that H_2 intravenous solution is safe for acute cerebral infarction, including patients treated with tissue−plasminogen activator.

Direct diffusion of hydrogen

H_2 can easily penetrate the skin and is easily distributed via blood flow throughout the body, so taking a warm H_2 bath is a method of incorporating H_2 into the body in daily life. Actually, H_2 bath powders are commercially available in Japan. Noda[23] discovered that H_2 delivery to cardiac grafts during cold preservation, efficiently ameliorated myocardial injury due to cold I/R. This new method for cold storage should be further developed for potential therapeutic during transplantation as well as operation.

CLINICAL RESEARCH AND APPLICATION

Respiratory system

Lung ischemia−reperfusion injury

Lung ischemia−reperfusion injury (LIRI) refers to the phenomenon that the lung has experienced a period of ischemia, and the ischemic damage is further aggravated after recovery blood perfusion. The mechanism of LIRI includes a significant involvement of ROS, intracellular calcium influx, endothelial cell injury, leukocyte sequestration and activation in the pulmonary circulation, activation of the complementary system, and the release of inflammatory mediators such as arachidonic acid metabolites[24]. Liu *et al*.[25] examined the effects of lung inflation with 3% H_2 during the cold ischemia phase of lung graft function in rats. They also found that H_2 has an antioxidant and anti−inflammatory influence on LIRI when it is inhaled by patients. The subsequent year, Meng *et al*.[26] found that H_2 combined with Carbon monoxide(CO) will enhance lung protection in LIRI models of rats.

Hyperoxic acute lung injury

Hyperoxic acute lung injury (HALI) refers to the damage to the lungs secondary to exposure to elevated oxygen partial pressure. It is a major clinical problem for patients undergoing supplemental oxygen therapy and has mostly been a concern in clinical practice with the development of deep diving[27]. ROS plays an important role in HALI as Sun´s study[28] found that H_2 could reduce HALI, as well as oxidative stress. Later, Sun´s team studied the mechanism of H_2 in rats, finding that H_2 could significantly reduce HALI by reducing lung edema and apoptosis, inhibiting the elevation of endoplasmic reticulum stress (ERS) and increasing SIRT1 expression. This indicated that H_2 reduced HALI related ERS and the mechanism may be associated with the upregulation of SIRT1.

Acute lung injury

Acute lung injury (ALI) is a complex clinical syndrome involved acute inflammation, microvascular damage, and increased pulmonary vascular and epithelial permeability, frequently resulting in acute respiratory failure culminating in fatal acute respiratory distress syndrome[29]. Ying[30] reported in 2017 that hydrogen water attenuated ALI induced by oleic acid in rats and might protect against ALI through selective antioxidation and inhibiting inflammatory infiltration. Audi[31] detected and tracked the antioxidant and antiapoptotic properties of H_2 therapy *in vivo* in as early as 24 h after hyperoxia exposure. The result showed that H_2 provided protection in rat models of ALI by inhalation. Also hydrogen saline may present a novel therapeutic approach for the treatment of ALI in Liu´s study[32], where it may aid in protecting against lung injury. While the underlying mechanism regarding the effect of hydrogen saline is unknown, Du[33] supposed the mechanism may be associated with hydrogen inhibiting the release of pro−inflammatory cytokines, promoting anti−inflammatory cytokine release, and reducing oxidative damage.

Nervous system

Intracerebral hemorrhage

Intracerebral hemorrhage (ICH) is a devastating stroke subtype with high mortality and morbidity rates. At present, no specific medical therapy is available to treat ICH. There is evidence to suggest that ROS damage the blood−brain barrier and increase brain injury. The •OH and peroxynitrites are active ROS that react indiscriminately with nucleic acids, lipids, and proteins, resulting in DNA fragmentation, lipid peroxidation, and protein inactivation[34]. Takeuchi[35] focused on the effects of medical treatment only using H_2 (without surgery) on ICH. The results showed H_2 depressed oxidative DNA damage in the brain, but unexpectedly had no beneficial effects on the brain edema, which means the mechanism of ROS in ICH may be more complicated than previ−

ously believed. Therefore further studies are required to investigate whether H_2 is effective for ICH.

Neuronal ischemia-reperfusion (I/R) injury

Acute neuronal injury during I/R has been attributed to loss of mitochondrial permeability transition coupled with mitochondrial dysfunction. Cui *et al*.[36] showed that hydrogen rich saline was able to attenuate neuronal I/R injury, probably by selectively reducing cytotoxic oxygen radical function in rats. Moreover, Zhou[37] found the beneficial effects of hydrogen rich saline against spinal cord I/R injury by reducing oxidative stress.

Neurodegenerative disorder

Neurodegenerative disorders, including mild cognitive impairment (MCI) and dementia attributes to oxidative stress. Nishimaki[38] assessed the effect of hydrogen rich saline on oxidative stress model mice and subjects with MCI. They found hydrogen rich water reduced oxidative stress markers and suppressed the decline of memory impairment and neurodegeneration. Dohi[39] also found that hydrogen rich saline reverses neurodegenerative changes induced by traumatic brain injury.

Carbon monoxide (CO) poisoning

Neuronal injury caused by acute CO poisoning is partly free radical induced. In a model of CO induced poisoning, rats treated with H_2-rich saline significantly reduced the generation of ROS and subsequent lipid peroxidation in the nerve cell, improving the cognitive deficits. The mechanism of this protection may be related to reducing oxidative injury by affecting cells *in vivo*[40].

Cardiovascular system

In heart failure induced by cardiac fibrosis attributing to sustained pressure overload, oxidative stress plays a significant role in cardiac remodeling and heart failure independently of etiological factors. Yang[41] observed that H_2 saline treatment improved interstitial fibrosis and cardiac function, decreased the level of ROS, the oxidative stress marker malondialdehyde(MDA) and the expression of nitrogen oxides(NOXs), while increasing the activity of the antioxidant enzyme SOD. These results indicate that H_2 saline can improve cardiac function by reducing interstitial fibrosis through its antioxidative fuctions. In addition, Wu[42] investigated the *preventive* effects of H_2 treatment on doxorubicin-induced heart failure in rats. The results showed that the plasma level of oxidative stress markers were decreased in animals treated with H_2 saline. Additionally, Shinbo[43] concluded that breathing H_2 plus NO may have beneficial effects for I/R injury in murine heart.

Digestive system

Xia *et al*.[44] reported that hydrogen-rich water significantly attenuated oxidative stress in chronic hepatitis B patients, but the effects of hydrogen-rich water on HBV DNA load and liver function has yet to be confirmed. In 2017, Ikeda *et al*.[45] discovered that hydrogen-rich water can down-regulate the oxidative stress marker malondialdehyde(MDA), which prevented intestinal dysbiosis and could potentially be a new therapeutic strategy in cases of critical disease. Based on the observation that acetaminophen induces hepatotoxicity in blood and liver samples, Zhang[46] discovered that hydrogen-rich water has significant therapeutic potential in inhibiting oxidative stress and promoting liver regeneration.

Other systems

In brief, H_2 has promising preventive and therapeutic applications in various systems. For example, hydrogen-rich water is effective in preventing acute hearing loss due to transient cochlear ischemia[47]. It has also been found to visibly improve liver ischemia-reperfusion injury[48]. In addition, hydrogen-containing gas (1.3% hydrogen + 20.8% oxygen + 77.9% nitrogen) pre-inhaled can alleviate radiation-induced skin injury[49].

Application in transfusion medicine

In 2014, our team demonstrated that H_2 had great efficacy and lack of adverse effects on platelet storage[50]. During storage, platelets experience a series of transformations in morphology, function, as well as inhibition, known as platelet storage lesions. These injuries lead to platelet apoptosis, a weakening of clinical efficacy and in advanced cases the rendering of transfusions ineffective. We added hydrogen to platelets which were stored at (22±2) ℃ and found hydrogen helped to maintain pH and reduce the expression of CD62p. Hydrogen gas may play an effective role in preserving platelets by inhibiting the denigration of platelets and maintaining the pH value in the plasma. While the mechanism has not been fully clarified, we suspect that it is related to antioxidation. Hydrogen is a promising agent for blood cell storage in transfusion medicine.

EXPECTATION

Hydrogen has wide application prospects in clinical research. The mechanism has not only antioxidant

property, but also anti−inflammatory, anti apoptotic, anti allergic and the ability to promote the energy metabolism. These functions are coordinated together. However, research on the mechanism of hydrogen is still only superficial and the molecular target of antioxidation is still unknown. We hope that the target of hydrogen can be found not only to serve patients undergoing clinical procedures, but also for the preservation of blood products.

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