Hydrogen applications: advances in the field of medical therapy

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Abstract

Hydrogen (H_2) has been widely used in the chemical industry as a reducing agent. As the researches move along, increasing attention has been paid to its biological functions. The selective antioxidant effect of hydrogen is considered to be the main reason for medical applications. So far, many studies have confirmed its potential protective effects on ischemia/reperfusion injury of multiple organs, neurodegenerative diseases, bone and joint diseases, and respiratory diseases, opening a new era in the medical research and application of H_2. Increasing studies have focused on its biological effects and molecular mechanisms in the treatment of different diseases. In this paper, we review the biological effects, molecular mechanisms and methods of H_2 supply. We do hope that the advances in materials science can be better translated into medical applications and solve clinical problems. The medical application of H_2 is promising, and how to prepare an H_2 sustained-release system to achieve a sustained and stable H_2 supply in the body and ultimately improve the therapeutic effect of H_2 is a problem worthy of further investigation.

Key words: anti-allergic reactions; anti-apoptotic effect; anti-inflammatory; antioxidant; biological effects; energy generation; hydrogen; molecular mechanisms

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Introduction

Hydrogen (H_2) is a colorless, odorless gas with a small molecular weight and the lightest weight, which consists of one electron and one proton.[1] It has a low solubility at room temperature under the normal atmospheric pressure and has not been widely used in the biomedical field due to its limited application technology.[2] In recent years, as scholars have conducted in-depth research on the reduction and anti-free radical effects of H_2, it has been found that H_2 can directly react with active free radicals in cells such as hydroxyl free radicals and peroxynitro group to inhibit oxidative stress, reduce inflammation, inhibit cell apoptosis and reduce fibrosis. At the same time, H_2 does not interfere with other normal metabolic pathways while exerting its antioxidant effect. There is no research showing that H_2 will affect the level of signal conduction and active oxygen in which it participates.[3-5] Therefore, the therapeutic effect of H_2 in various fields has been paid seriousattention. With the advancement of gas extraction technology and the development of multidisciplinary comprehensive treatment, the therapeutic effect of H_2 has once again been included in extensive research by researchers. As early as 1975, high concentration H_2 was first published in the journal *Science* for the treatment of hairless albinism mice skin cancer model, which proposed that H_2 could scavenge hydroxyl free radicals.[6] As a signal transduction participant, H_2 may also regulate the expression of related proteins or the phosphorylation of certain signaling proteins.[7] With the deepening of research, H_2 has found an increasingly wide application in many fields, however, the toxic and side effects of H_2 on the body have not yet been discovered, so it has a broad application mechanism in clinical practice. Ohsawa et al.[8] proposed in 2007 that H_2 could reduce cerebral ischemia/reperfusion injury in rats by scavenging hydroxyl radicals, opening a new chapter in the field of H_2 medical research and application. Since then, the use of H_2 as a therapeutic gas has been widely studied. This article summarizes the application of H_2 in the medical field, which found the common challenge is how to maintain the effective concentration of H_2 in the body during the application of H_2 therapy. This suggests that the development of H_2 sustained-release system is expected to improve the therapeutic application value of H_2.

The literature search of the following databases was conducted: PubMed, Medline, and Google Scholar. The search terms used in various combinations included "hydrogen," "antioxidant," "anti-inflammatory," "anti-apoptotic effect," "anti-allergic reactions," "H_2-rich water," "H_2-rich saline," and the retrieval time was between 2011 and 2021; some literatures were retrieved according to the contents involved in the previous reference.

Biological Effects of Hydrogen

Anti-oxidation effect

As a reducing gas, H_2 has obvious advantages in anti-oxidation. H_2 can diffuse rapidly to reach the dangerous area, selectively binding and scavenging toxic hydroxyl radicals and nitrite anions, and does not affect biologically active free radicals such as H_2 peroxide.[9] Ono et al.[10] found that rats with acute cerebral ischemia and infarction could significantly improve cerebral

ischemia/reperfusion injury by inhaling 3% H_2, and the treatment was based on the selective scavenging of reactive oxygen species (ROS) by the antioxidant effect of H_2. Atherosclerosis is a multifactorial, long-term disease that can affect multiple organ functions, in which strong oxidative stress and inflammatory response are the main manifestations.[11] Its progress is closely related to the binding of oxidized low-density lipoprotein to cell surface receptor lipid peroxidase-1, which affects the normal function of endothelial cells. Studies[12-14] have shown that H_2 can reduce the level of oxidative stress in the aorta, decrease the production of oxidized low-density lipoproteins in the body, inhibit tumor necrosis factor α and nuclear factor κB (NF-κB) pathways while maintaining the normal vascular endothelial function, and reduce extracellular signals that regulate enzymes. Upstream Kinase phosphorylation inhibits Ras extracellular signal regulation pathway and blocks the G1-S cell cycle process, thereby inhibiting vascular intimal hyperplasia and delaying the development of atherosclerosis. Zhang et al.[15] indicated that H_2 gas inhalation improves lung function and protects established airway inflammation in the allergic asthmatic mice model, and they found it is associated with the inhibition of oxidative stress process. Hyspler et al.[16] conducted *in vitro* studies combining H_2 isotopes to confirm that H_2 has no damage to the body and can produce obvious antioxidant effects, which provides a theoretical basis for the corresponding H_2 research and application.

Anti-inflammatory effects

Gharib et al.[17] demonstrated the anti-inflammatory effect of H_2 for the first time. Their study indicated that breathing 0.7 MPa H_2 for 2 weeks could treat hepatitis caused by parasitic infection. However, due to the difficulty in the application of high-pressure H_2 in clinical treatment, it did not arouse wide attention. In recent years, related studies[18,19] have shown that H_2 under normal pressure can also slow down the inflammatory response induced by concanavalin, dextran sulfate, lipopolysaccharide, and zymosan in animal models, and has obtained good results. Huang et al.[20] found that mixing 2% H_2 during mechanical ventilation can reduce pulmonary interstitial edema, relieve alveolar cell membrane thickness and inflammatory cell infiltration, and effectively alleviate lung injury caused by ventilators. Kawamura et al.[21] in the study of rat lung transplantation model found that inhalation of 2% H_2 during surgery can block the pro-inflammatory mediators produced by lung surfactant, reduce apoptosis of B lymphocytes, mitigate lung injury and improve lung function decline. Rheumatoid arthritis is a chronic inflammatory disease characterized by the destruction of bone and cartilage.[22] Ishibashi et al.[23] found that drinking H_2-rich water (water containing saturated H_2) could reduce the oxidative stress caused by inflammatory factors, regulate the expressions of NF-κB and tumor necrosis factor α, and significantly improve the inflammatory cytokine expression of rheumatoid joints. These phenomena indicate that the basis of H_2 treatment of certain diseases is the existence of indirect molecular effects, which may be regulated by the activity of related proteins or enzymes, affecting the activity of signal transduction pathways, and thereby exerting the effect of controlling inflammation.

Anti-apoptotic effect

Caspases are a family of cysteine proteases that play essential roles in apoptosis (programmed cell death), necrosis, and inflammation.[24,25] Normally, caspase is in the non-activated state. The neuronal apoptosis program is activated by triggering the activation of caspase. cascade reaction of apoptotic protease and irreversible apoptosis occurs, which is mainly manifested by irreversible damage of brain tissue. In brain trauma models, early intraperitoneal injection of saturated H_2-rich saline can effectively reduce the level of malondialdehyde, down-regulate the expression of Caspase-3/12, reduce neuronal apoptosis and reduce cerebral edema.[26] Meanwhile, apoptotic protein Bas and Caspase-3 can be down-regulated, and the level of anti-apoptotic protein Bcl-2 can be up-regulated to reduce the nerve function injury. Chen et al.[27] found that H_2 can exert anti-apoptotic properties by inhibiting Caspase-3. NF-κB is a rapid transcription factor widely found in the cytoplasm, mainly involved in cell differentiation and apoptosis, as well as information transmission in the process of tumor growth inhibition. Zhang et al.[28] found that oral administration of saline containing saturated H_2 for one week in rats could effectively relieve acute peritonitis, and its protective effect might be related to the reduction of NF-κB activity and the inhibition of inflammatory response and oxidative stress. Alzheimer's disease is a neurodegenerative disease in which the loss of antioxidant defense enzyme function and the increase in ROS and reactive nitrogen species cause oxidation stress plays an important role in the formation of Alzheimer's disease. In animal models of β-amyloid-induced Alzheimer's disease, H_2 can inhibit c-Jun N-terminal kinase, NF-κB and other pro-apoptotic molecules, thereby reducing oxidative stress and playing a therapeutic role.[29]

Promoting energy generation

The wild type of obese db/db mice lacking functional leptin receptors can induce fatty liver through a high-fat diet.[30] Kamimura et al.[31] found that H_2-enriched water could significantly improve the fatty liver of db/db mice, and long-term consumption could significantly control the fat and body weight, and lower plasma glucose, insulin and triglyceride levels. The function of fibroblast growth factor 21 is to stimulate fatty acids and consume glucose and promote the recognition of E-linkage.[32] Gene expression profiles show that H_2-rich water can increase the expression of fibroblast growth factor 21.[32] Song et al.[33] reported that H_2-rich water can increase the expression of fibroblast growth factor 21, and prevent the metabolic syndrome by reducing serum low-density lipoprotein cholesterol level and improving the function of high-density lipoprotein.

Anti-allergic reactions

Itoh et al.[34] found that the oral intake of H_2-rich water abolishes an immediate-type allergic reaction in mice. In the mice RBL-2H3 mast cell model, they observed that drinking H_2-rich water inhibits neutral lysine phosphorylation and downstream molecular signaling, and subsequently inhibits activation of NADPH oxidase enzyme and ROS production, thereby slowing down skin allergic reactions. This suggests that H_2 plays a role in acute allergic reactions in mice by regulating specific signaling pathways, rather than scavenging

free radical activity. Other researches[15,35-37] have also pointed out that H_2 attenuates allergic inflammation by reversing the energy metabolic pathway switch. The occurrence of allergic airway inflammation is closely related to energy metabolism pathways. H_2 can regulate these energy metabolism pathways through a variety of mechanisms, directly inhibit the activity of glycolytic enzyme and stimulate the activity of mitochondrial oxidative phosphorylation enzyme, and ultimately reverse the up-regulation of glycolytic enzyme activity and the down-regulation of oxidative phosphorylation enzyme activity completes the switch of energy metabolism pathway, thereby reducing allergic airway inflammation.[38]

Molecular Mechanism of Hydrogen

Hydroxyl radical reduction

Hydroxyl radical (–OH) is one of the main initiators of free radical chain reaction.[39,40] Once the chain reaction starts in biofilm, it will spread continuously and cause serious damage to tissue cells. The chain reaction produces lipid peroxides and oxidative stress markers, such as 4-hydroxynonenal and malondialdehyde. Venkata Mohan et al.[41] noted that H_2 can reduce the levels of these oxidation markers. The distribution of H_2 in the lipid phase of the biofilm is higher than that of the water phase, and unsaturated lipid area is the main target of the initial chain reaction, so H_2 can directly inhibit the chain reaction to avoid cell damage. In addition, hydroxyl radicals can modify deoxyguanosine to form 8-hydroxydeoxyguanosine. Animal and clinical studies[42,43] have shown that H_2 can also reduce 8-hydroxydeoxyguanosine levels, and H_2 has been shown to reduce hydroxyl radicals in cell experiments. Moreover, related experiment[44] confirmed that H_2-rich saline can neutralize the hydroxyl free radicals caused by ionizing radiation, reduce the loss of male germ cells in mice induced by radiation.

Reduction of nitrite

Protein tyrosine nitration is an important selective translational modification, and the product, 3-nitrotyrosine has had been utilized as a biomarker of tissue and cell injury, which is an important marker of oxidative stress in the body.[45] Studies[46-48] have shown that H_2, H_2-rich water or H_2-rich saline can reduce the level of nitrotyrosine in animals. Ishibashi et al.[49] reported that drinking H_2-rich water can reduce the level of nitrotyrosine in patients with rheumatoid arthritis. It is known that many protein factors involved in the regulation of nitrotyrosine protein transcription are modified by -O-NO_2 or -S-NO_2. Therefore, H_2 may be reduced by -O-NO_2 or -S-NO_2 to regulate the expression of various protein factors, thereby reducing the production of nitrotyrosine protein.50

Regulating endogenous antioxidant pathways

Nuclear factor erythroid-related factor 2 (Nrf2)-antioxidant response element is one of the most important defensive transduction pathways against internal and external oxidative stress.[51] Nrf2 is a molecule that manages and controls the endogenous antioxidant system. It can combat oxidative stress and various toxic substances by inducing the expression of related proteins, such as hemeoxygenase-1.[52] Due to its endogenous characteristics, studying the regulation of H_2 on it may open up a new way to realize effective and controllable antioxidation therapy. The treatment of lung diseases with high oxygen concentration in critically ill patients is the main cause of hyperoxic lung injury.[53] Kawamura et al.[54] reported that normal mice can develop lung injury after 60 hours of exposure to high concentrations (98%) of oxygen. Inhalation of 2% H_2 can up-regulate genes related to Nrf2, increase mRNA transcription and hemeoxygenase-1 protein expression, and improve oxygen lung injury. However, H_2 did not alleviate hyperoxic lung injury or induce HO-1 in Nrf2-mice, suggesting that H_2 could improve hyperoxic lung injury by inducing the expression of Nrf2-related factors (such as hemeoxygenase-1). Lactulose can be fermented by bacteria in the gastrointestinal tract to produce a large amount of H_2.[55] Orating lactulose can activate the expression of Nrf2 in the brain, and has a certain alleviating effect on cerebral ischemia/reperfusion injury in rats. Beside the protein factors mentioned above, the body itself has a variety of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, etc. Studies[56,57] have shown that H_2 can also reduce oxidative stress damage by increasing the activity of endogenous antioxidant enzymes. Therefore, H_2 reduces oxidative stress not only through direct action but also indirect action, that is, by inducing the endogenous antioxidant system, H_2 indirectly plays an antioxidant effect and regulates the expression of pro-inflammatory and pro-apoptotic factors. H_2 can counteract different pathological conditions by up-regulating or down-regulating the expression of related proteins indirectly.

In most inflammatory models, H_2 plays an anti-inflammatory role by reducing the expression of pro-inflammatory cytokines such as NF-κB, tumor necrosis factor α, interleukin (IL)-1, IL-6, IL-12, C-C motif chemokine ligand 21, interferon-γ, intercellular adhesion molecule-1, prostaglandin E2, and high mobility group box 1.[58,59] Furthermore, H_2 can also play an anti-apoptotic effect by up-regulating or down-regulating pro-apoptotic factors. Several studies have reported that H_2 can promote the expression of anti-apoptotic factors such as Bcl-2 and Bcl-XL, and inhibit the expression of apoptotic proteins such as Caspase-3, Caspase-8 and Caspase-12.[49,60-62] In the expression of pro-apoptotic factor Bax, H_2 can not only inhibit the transcription and translation of the genes it encodes, but also inhibit the Bax protein from the cytoplasm.

Acting as an adrenal receptor agonist

The therapeutic potential of molecular H_2 is emerging in several human diseases and in their animal models, including in particular Parkinson's disease. H_2 supplementation of drinking water has been shown to exert disease-modifying effects in Parkinson's disease patients and neuroprotective effects in experimental Parkinson's disease model mice.[63] Research by Matsumoto et al.[64] found that H_2 gas can increase the expression and secretion of ghrelin. After the hormone enters the brain, it can protect dopamine neurons by activating receptors on dopamine neuron cells and protect dopamine neurons from toxin damage. However, this effect can be disrupted by B1 adrenergic receptor blockers or ghrelin receptor blockers, which may be a new explanation for the protective effect of H_2, that is, H_2 activates the B1 adrenal receptors in the stomach,

thereby promoting the synthesis and release of ghrelin and producing the neuroprotective effect.

Suppression of Wnt/β-catenin signal

The Wnt/β-catenin signaling pathway is a molecular system involved in regulating cell division, growth, and maintaining the stability of tissue cells.[65] In some inflammatory diseases and tumor cells, the Wnt/β-catenin system is over-activated and the β-catenin level stays elevated. The latest studies[7,66,67] reveal that H_2 can inhibit the activated Wnt/β-catenin signal by promoting the degradation of β-catenin and accelerating its phosphorylation. H_2 action had no effect on the normal physiological level of Wnt/β-catenin, and only showed corresponding manifestations when Wnt/β catenin was abnormally activated, which also reflects the active therapeutic effect of H_2 in many diseases. Abnormal activation of Wnt/β-catenin signal is also a manifestation of worsening osteoarthritis, so Wnt/β-catenin signaling pathway may be a therapeutic target for osteoarthritis. In 2016, researchers discovered for the first time in human osteoarthritis chondrocytes that H_2 can not only inhibit Wnt/β-catenin signaling but also inhibit the loss of proteoglycan in ATDC5 chondrogenic cells induced by Wnt3a, 6-bromoindirubin-3'-oxime.[7] Meanwhile, studies have also found that oral H_2-rich water or local injection of H_2 sustained-release system into the knee joint can delay the development of knee osteoarthritis.[66,68]

Regulating other signaling pathways

Itoh et al.[69] found that in the mouse model of type 1 hypersensitivity, taking H_2-rich water can slow down the passive cutaneous anaphylaxis and reduce the level of plasma histamine. H_2pretreatment of RBL-2H3 cells sensitized to IgE basophilic leukemia could reduce the release of β-aminohexidase, a threshing marker. Moreover, H2 can inhibit the phosphorylation of neutral lysine and its downstream signaling molecules Syk, phosphoinositide phospholipase Cγ1, phosphoinositide phospholipase Cγ2, Akt, extracellular signal-regulated protein kinase 1/2, c-Jun N-terminal kinase, p38 and cytosolic phospholipase A2.7 In addition, H_2 also inhibits the production of antigen-induced activated NADPH oxidase enzyme and ROS, which is believed to be a result of signal transduction inhibition.[70] The above analysis shows that H_2 does not scavenge free radicals directly, but acts as a specific signal regulator to improve type 1 hypersensitivity. Tanaka et al.[71] reported that the introduction of H_2 during lung transplantation can significantly increase the expression of surfactant-related molecules, ATP synthase and stress response molecules. Li et al.[72] indicated that H_2 can reduce the expression level of specific markers of osteoclasts, including tartrate-resistant acid phosphatase, calcitonin, cathepsin K, matrix metalloproteinase-9, carbonosidase type II weaving protein K, matrix Golden vacuole H^+-ATPase, etc.

H_2 can improve various pathological conditions by regulating the expression of various proteins, which include: matrix metalloproteinase, brain natriuretic peptide, intercellular adhesion molecule-1, myeloperoxidase, cyclooxygenase-2, neuronal nitric oxide synthase, manganese superoxide dismutase, collagen III, ionized calcium binding adapter molecule 1.[73,74] These molecules may not be the main target molecules of H_2, but their indirect function can affect the termination effect, which can be analyzed at the gene and protein level to find molecular targets for H_2 to prevent and treat diseases.

The Ways of Hydrogen Supply in Medical Research

Direct use of H_2

Inhalation of H_2 gas

H_2 was first applied in the field of diving, mainly in order to increase the diving depth and avoid nitrogen anesthesia, H_2 and oxygen mixture was selected as the respiratory medium.[75] This move was not intended to treat disease but is the first to demonstrate the safety of inhaling H_2. Currently, studies have shown that H_2/oxygen mixed gas inhalation improves disease severity and dyspnea in patients with coronavirus disease 2019 in a recent multicenter, open-label clinical trial, and the safety profiles have rendered H_2-oxygen inhalation particularly suitable for relieving dyspnea and other respiratory symptoms in patients with coronavirus disease 2019, regardless of the disease severity.[76,77] What's more, physiological adverse reactions caused by H_2 inhalation have not been observed, and the inhalation of H_2 does not affect physiological parameters (pH, blood pressure, oxygen partial pressure, and body temperature). Studies on various disease models have shown that low concentrations of H_2 (2–4%) can effectively improve Parkinson's disease, ventilator-related lung injury, and lung injury induced by hyperoxia.[29,43,63] Inhalation of H_2 gas abrogated ovalbumin-induced increase in lung resistance has been confirmed in rats, and H_2 gas can reduce the number of total cells, eosinophils and lymphocytes in bronchial alveolar lavage fluid. H_2 gas inhalation improves lung function and protects established airway inflammation in the allergic asthmatic mouse model, which may be associated with the inhibition of oxidative stress process and provides a potential alternative therapeutic opportunity for the clinical management of asthma.[15] Buchholz et al.[78] used an aesthesia liquid gasifier to allow mice to suck 2% H_2 with a mask, which proved that H_2 could reduce the up-regulation of inflammatory chemokine ligand 2 and tumor necrosis factor α induced by graft, and significantly decrease the lipid peroxidation and neutrophils recruitment. Fukuda et al.[79] administered H_2-containing gas to the anesthetized mice: 10 minutes before reperfusion, H_2 was supplied to the anesthetic gas at a flow rate of 1 L/min through a gas flowmeter until the end of reperfusion. The gas flow meter controls the flow, and the respiratory gas analyzer measures the H_2 concentration, which proves that H_2 can reduce oxidative stress. It can be seen that the study of inhalation of H_2 needs to design the corresponding combination of inhaled gas components and inhalation equipment and choose the H_2 concentration reasonably. Therefore, the inhalation of H_2 research needs to design the corresponding combination of inhaled gas composition, specific equipment, and reasonable selection of H_2 concentration. It is also worth noting that, if the H_2-oxygen ratio imbalance, it is prone to explosion hazard, which requires the H_2 concentration controlled within the safety margin below 4% or above 75%.[80,81]

Abdominal injection

Although oral administration seems safe and convenient, H_2 in water is difficult to dissolve and evaporates over time, and some H_2 is lost in the stomach or intestines during oral administration, making it difficult to control the concentration of H_2 administered. Administration of H_2 via an injectable H_2-rich vehicle may allow delivery of more accurate concentrations of H_2.[82] Liu et al.[83] compared intraperitoneal injection of H_2, H_2-rich water and intravenous injection of H_2-rich water, and found that intraperitoneal injection of H_2 can maintain H_2 levels for a longer time. Wang et al.[84] established a hindlimb ischemia/reperfusion model, and randomly divided 30 rabbits into sham operation group, ischemia/reperfusion group, and ischemia/reperfusion combined with H_2 perfusion group. The ischemia/reperfusion combined with H_2 perfusion group was intraperitoneally injected with H_2, while the sham operation group and the ischemia/reperfusion groupwere intraperitoneally injected with the same amount of air. They found that the intraperitoneal injection of H_2 can reduce serum propylene glycol levels and skeletal muscle inducible nitric oxide synthase and increase endothelial nitric oxide synthase expression levels.[85,86] So, H_2 has a protective effect on skeletal muscle injury induced by ischemia/reperfusion.

H_2-rich liquid

Oral H_2-rich water

Oral H_2-rich water can effectively avoid the potential unsafety of H_2 inhalation. The commonly used preparation methods are as follows.[87-90] (1) Electrolytic H_2: H_2 and oxygen are produced simultaneously by electrolysis of water. The saturation concentration of H_2 in water is about 1.6 ppm (1 ppm = 1 mg/L). (2) Three-dimensional structure of nanoporous magnesium is prepared by a physical vapor deposition method, which can generate H_2 with salt water directly, and H_2 generation property of nanoporous magnesium is better than present materials. When the water flows through the filter element, H_2 is produced through a replacement reaction. However, with the increase of usage, the filter element is oxidized and its H_2 production capacity decreases. (3) High pressure dissolution method. High concentration of H_2 is dissolved in water and sealed under high pressure and special process. H_2 concentration can reach more than 3ppm under high pressure. Nishimaki et al.[91] found that drinking saturated H_2 water can reduce oxidative stress and improve the cognitive function of dementia mice. Muramatsu et al.[50] established a newborn rat bronchopulmonary dysplasia model by injecting lipopolysaccharide into the amniotic fluid of pregnant rats, their study showed that pregnant rats drinking H_2-rich water can reduce the content of ROS in their offspring and significantly improve bronchopulmonary dysplasia. Matsumoto et al.[64] found that drinking H_2-rich water could increase the secretion of ghrelin and improve the loss of dopamine neurons in basal ganglia in Parkinson's mouse model. Furthermore, compared with other H_2 supply modes, drinking H_2-rich water can significantly increase the local H_2 concentration in the gastrointestinal system, which may be more suitable for gastrointestinal diseases research. In addition, drinking H_2-rich water can also reduce the scores of the Alzheimer's disease assessment scale-cognitive section of patients with mild cognitive impairment, bring down blood lactic acid levels after high-intensity exercise and improve the ratings of perceived exertion, ameliorate lipid metabolism disorders in patients with metabolic syndrome, regulate lipid metabolism in patients with metabolic syndrome, and improve the liver function of patients with colorectal cancer undergoing chemotherapy.[92-95]

H_2-rich saline for injection

The preparation method of H_2-rich 0.9% sodium chloride solution is similar to that of H_2-rich water, but the concentration is generally lower than that of oral H_2-rich water, which is about 1 ppm.[93] Compared with oral H_2-rich water, H_2-rich saline for injection is easier to control the concentration of H_2. It is also low cost, safe and convenient operation, high efficiency and utilization rate. The injection methods include intraperitoneal, intravenous and intrathecal injection, and intraperitoneal injection is the most widely used.[94-96] In doxorubicin-induced cardiomyopathy rat models, intraperitoneal injection of H_2-rich saline improved cardiac function and alleviated inflammatory cell infiltration and focal myolysis in myocardial tissue, greatly enhancing the rat survival rate.[97] Ogawa et al.[98] constructed an ischemic hearing loss model by clamping bilateral vertebral arteries of gerbils for 15 minutes followed by intravenous injection of 5 mL sodium H_2-rich solution. It was found that H_2 could reduce cochlear injury of gerbils and partially restore their hearing.

H_2-rich saline for infiltration

The cold ischemia/reperfusion injury of transplanted organs can be improved by the organ refrigerated solution enriched with H_2. Uto et al.[99] found that the H_2-rich University of Wisconsin solution could reduce oxidative stress injury and hepatocyte apoptosis in rats with liver transplantation. H_2-rich University of Wisconsin solution can prolong the graft preservation time and improve the graft function. Takahashi et al.[100] induced lung ischemia/reperfusion injury by clamping the left hilum of rats for 1 hour and reperfusion for 3 hours. In their study, 0.9% sodium chloride solution or H_2-rich 0.9% sodium chloride solution was injected into the left thoracic cavity of rats during pulmonary ischemia, and H_2 concentration in the left lung was measured by gas chromatograph. The results turned out that the lung function of the H_2-rich saline group was significantly better than that of the saline group. Meanwhile, the level of pro-inflammatory factors was significantly lower. H_2-loaded eye drops open a precedent for topical H_2 medication.[101] Since H_2 can penetrate the skin and spread around through the bloodstream, so it can be used for local infiltration.[102] However, due to its low solubility and uncontrollable concentration, the relevant studies can only be qualitative but not quantitative.

H_2 microbubbles

Due to the low solubility of H_2, it is difficult for it to be released directionally either by direct inhalation or in liquid solution, which limits its application to a certain extent. Ultrasonic microbubble drug delivery technology can make up for this defect. Drug-loading ultrasound microbubbles generally use lipid microbubbles that have good targeting properties and maintain drug stability in theranostic formulations. Its

chemical composition and structure determine microbubbles' acoustic properties, stability, drug loading capacity and *in vivo* behavior.[103] What's more, it is also convenient for ultrasound contrast inspection. The preparation method of H_2 drug-loaded microbubbles is similar to that of traditional lipid drug-loading, and finally forms perfluoropropane/H_2 microbubbles mixed in a certain proportion.[104] Li et al.[105] indicated that for a solution with a concentration of (1.14 ± 0.07) × 100 microbubbles/mL, the peak time of H_2 released by microbubbles is 80 seconds, and the H_2 content in the solution can be maintained at 33.45 ± 0.67 ppm, which is much higher than conventional H_2-rich water. Zhang et al.[106] studied the directional release of H_2-carrying ultrasound microbubbles in mouse ischemia/reperfusion myocardium. H_2-carrying microbubbles were injected through the caudal vein, and ultrasound was used to detect the left ventricular angiography imaging, results confirmed that H_2 microbubbles played a protective role in myocardial ischemia/reperfusion injury. At the same time, new ideas for ultrasound-guided gas visualization therapy were proposed. The well-targeting property can improve the bioavailability, permeability and controlled release, and can also be observed by ultrasound. However, it is just the underway step on studying and still needs further investigation.

Promotion of endogenous H_2 production

In addition to the aforementioned exogenous H_2 supply pathway, stimulating the production of endogenous H_2 can also achieve the purpose of H_2 supply.[107] The endogenous H_2 in the human body comes from the glycolysis of bacteria in the body, mainly from the intestines. It is convenient to administer and produces H_2 quickly. Specific methods include oral drugs (acarbose, fructose, etc.) and diet promotion (turmeric, milk, etc.).[108] Due to individual differences in the intestinal flora, lack of quantitative experiments and the fact that H_2 can only diffuse from the intestine, many of the above variables need to be considered when choosing this method for H_2 supply. Ishida et al.[109] showed that oral administration of fructo-oligosaccharides could significantly increase the concentration of H_2 in the abdominal cavity and portal vein of rats. The concentration of H_2 in the abdominal tissues (especially the adipose tissues) of the oral oligosaccharide group was 5.6–43 times higher than that of the control group, while the concentration of H_2 in the abdominal tissues was 11 times higher than that of the control group. The NF-κB, IL-6 and C-C motif chemokine ligand 2 gene expression levels in adipose tissue were decreased after continuous feeding of oligosaccharide or inulin for 28 days in rats on a high-fat diet, suggesting that oral oligosaccharide may promote endogenous H_2 production and improve metabolic syndrome through anti-inflammatory effects.[110] Oral lactulose can also promote the production of H_2 by bacteria in the intestinal. After 70 minutes of oral administration of 6 g of lactulose in healthy people, the exhaled H_2 concentration began to gradually increase and can be as high as 42 ppm after 180 minutes. However, there were some individual differences, among which 14% of healthy people showed no significant changes after oral lactulose. Oral lactulose has been found to promote intestinal tissue repair in mice with colitis and reduce the levels of inflammatory factors.[111] This protective effect can be blocked by antibiotics that inhibit the intestinal flora. In addition, drinking milk can delay the time to reach the peak of the expiratory H_2 content than drinking H_2-rich water, and can maintain a high expiratory H_2 content for a long time. Endogenous H_2 is also affected by physiological conditions.[112] Studies[113,114] have found that expiratory H_2 content increases when experimental animals are dehydrated. Therefore, further studies on endogenous H_2 need to consider lactose and fructose intolerance, physiological conditions and individual differences in the intestinal flora.

Conclusions

Various modes of H_2 supply and their mechanisms of action have been studied, but the efficacy and safety of the treatment still need to be further clarified by a large number of prospective clinical studies. There are still many problems to be solved in actual clinical applications. For example, how to choose suitable H_2 supply methods for different diseases, the effective H_2 concentration required by different H_2 supply methods, the peak speed, duration, and dosage. In addition, there are few quantitative studies on the concentration of H_2 in H_2-containing solutions, and because H_2 has low solubility and volatilization problems, how to increase and maintain the concentration of H_2-containing solutions has become a major problem, which is expected to be solved by nanotechnology.

Author contributions
TY was responsible for collecting literature and writing manuscripts; JNZ and NRB contributed to polish the language and responsible for final version for this commentary.
Conflicts of interest
The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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