

The transfer of hydrogen from inert gas to therapeutic gas

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Abstract

Hydrogen is the most abundant chemical element in the universe, and has been used as an inert gas for a long time. More recent studies have shown that molecular hydrogen as a kind of antioxidant, anti-inflammatory, anti-apoptosis, gene expression and signal modulation molecule, can be used for the treatment of many diseases. This review mainly focuses on the research progresses of hydrogen in various medical fields and the possible action mechanisms.

Key words: hydrogen; action mechanisms; antioxidant; gene expression; signal-transduction; injection methods; research progresses; future direction

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INTRODUCTION

Hydrogen was used in the diving medical field at the very beginning, and the main therapeutic effect was ascribed to the inert nature in the hyperbaric environment but not its biological effect. Until the year of 1975, Dole et al.¹ demonstrated the therapeutic effect of hydrogen in a hairless albino mouse model of squamous cell carcinoma. Subsequently, Ohsawa et al.² reported that hydrogen could exert its antioxidant property by selectively reducing hydroxyl radicals ($\bullet\text{OH}$). After that, hydrogen as a promising therapeutic gas has been proven beneficial in many disorders, but the specific mechanisms of action have not been fully clarified, which need to be further explored.

THE POSSIBLE ACTION MECHANISMS OF HYDROGEN

The acute antioxidant effect of hydrogen

Researchers speculated that hydrogen could exert its acute effect by two possible mechanisms as described in previous studies. First, hydrogen could neutralize $\bullet\text{OH}$ caused by ionizing radiation,³ and the eye drops containing hydrogen decreased the $\bullet\text{OH}$ level caused by retinal ischemia-reperfusion injury,⁴ which indicated that hydrogen could play an efficient role by clearing $\bullet\text{OH}$ directly; secondly, studies have shown that hydrogen could decrease the level

of nitrotyrosine in various animal models,⁵⁻⁷ the generation of which was performed by the conjugation of nitroso anion and tyrosine residues. Thus we could attribute the antioxidant effect of hydrogen to the partial reduction of nitrite.

The chronic regulation mechanisms of hydrogen

It is becoming clearer that hydrogen has not only acute antioxidant effect but also a chronic regulating effect of gene expression and signal transduction. Our group found hydrogen inhibited the expression of miR-200 family in hypoxia/reoxygenation-induced HT-22 cells.⁸ Some researchers showed that hydrogen could regulate the lipopolysaccharide-activated signaling pathway at the posttranscriptional level, including microRNAs and their target proteins, and thus decreased the diabetic retinal injury.⁹ In addition, hydrogen also exerted its beneficial effect by inhibiting the expression of nuclear factor-kappa B (NF- κB)-regulated gene, and attenuating extracellular regulated protein kinases (ERK), p38 mitogen-activated protein kinase (p38 MAPK), and NF- κB signaling in the healthy mouse liver.¹⁰

THE INJECTION METHODS OF HYDROGEN

According to previous studies, the most common injection methods of hydrogen included inhaling hydrogen gas,



intravenous or intraperitoneal injecting hydrogen solution, and drinking hydrogen rich water (HRW).¹¹⁻¹³ However, the above methods may not be able to guarantee enough hydrogen concentration in certain local damage models. Shigeta et al.¹⁴ demonstrated the luminal injection of hydrogen-rich glucose saline may be a novel and promising way in a small bowel ischemic/reperfusion (I/R) injury model. In addition, subcutaneous injection of hydrogen gas could also be used to verify the beneficial effects of hydrogen in a type 2 diabetes mellitus (T2DM) mouse model.¹⁵ Another study demonstrated the usefulness of hydrogen in phacoemulsification by 24-hour and 1-week exposure of the ocular irrigating solution to 100% hydrogen gas in an acrylic chamber.¹⁶ Subarachnoid cavity injection of hydrogen rich saline (HRS) has also become a new treatment for brain and spinal cord diseases.¹⁷

THE RESEARCH PROGRESSES OF HYDROGEN

Hydrogen and ear, nose and throat diseases

Eye diseases

The mechanical injury and local temperature increase induced by phacoemulsification may result in the damage of corneal endothelial cells and even permanent visual impairment, but hydrogen dissolved in the ocular irrigating solution could significantly protect corneal endothelial cells from phacoemulsification-induced oxidative stress and damage.¹⁶ Kubota et al.¹⁸ reported hydrogen-enriched irrigation solution could be a novel and promising therapy for angiogenesis in cornea and prevent blindness caused by alkali burn, which was triggered by excessive production of reactive oxygen species (ROS). Moreover, postconditioning with inhaled 67% hydrogen appeared to promote the survival of retinal ganglion cells and preserve visual function induced by retinal I/R injury *via* anti-oxidative, anti-inflammatory and anti-apoptosis pathways.¹⁹

Otology diseases

Cisplatin is a widely used chemotherapeutic agent for the treatment of various malignancies, and may produce ototoxicity by activating enzymes specific to the cochlea.²⁰ It has been reported that inhalation of hydrogen gas significantly increased the numbers of remaining auditory hair cells and reduced the level of •OH induced by cisplatin without compromising the anti-tumor effect.²¹ HRS could also alleviate experimental noise-induced hearing loss, partially by preventing the death of cochlear hair cells after intensive noise exposure.²²

Oral diseases

Researchers investigated the effects of HRW on aging periodontal tissues and found HRW alleviated periodontal

oxidative damage but did not suppress inflammatory reactions.²³ A recent pilot study compared the effects of non-surgical periodontal treatment with or without drinking HRW on periodontitis, which indicated that HRW group showed stronger antioxidant capacity and greater improvements in probing pocket depth and clinical attachment level than the control group.²⁴ In addition, researchers also reported that HRW intake reduced oxidative stress and inflammatory response, and accelerated oral palatal wound healing *via* activation of the Nrf2/antioxidant defense pathway.²⁵

Nasal diseases

In a recent study, HRS reduced the frequency of sneezing and scratches in guinea pigs of allergic rhinitis accompanying with a decrease of eosinophils cells in blood and eotaxin in nasal mucosa.²⁶ Moreover, HRS may also inhibit the inflammatory responses and increase the ratio of CD4(+) CD25(+) Foxp3(+) Treg cells of peripheral blood in allergic rhinitis guinea pigs.^{27,28}

The therapeutic effect of hydrogen on nervous system diseases

Neurodegenerative diseases

Because of the ability of HRS to cross the blood-brain barrier (BBB), it was supposed hydrogen had therapeutic potentials in neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis. Lin et al.²⁹ found that HRW could exert its protective effect by stimulating AMP-activated protein kinase (AMPK) in a sirtuin 1-dependent pathway, which up-regulated forkhead box protein O3a downstream antioxidant response and diminished amyloid β -induced mitochondrial potential loss. Matsumoto et al.³⁰ pointed out the neuroprotective effect of HRW may result from gastric induction of ghrelin and the subsequent activation of ghrelin receptors in a PD mouse model, however, in another ghrelin-knockout PD mouse model, researchers found ghrelin may not be the only factor for hydrogen-induced neuroprotection.³¹ Oxidative stress contributed to the pathology of amyotrophic lateral sclerosis, but classical antioxidants such as vitamin C and vitamin E could not work well in clinical trials. A recent research showed the protective effect of HRS in a mutant SOD1 G93A transgenic mouse model, which presented with attenuated loss of motor neurons, declined oxidative stress related index and prolonged survival.³²

Traumatic brain injury

A study reported in 2012 found that HRS administration after mild traumatic brain injury significantly elevated the levels of silent information regulator 2 and molecules associated with brain-derived neurotrophic factor mediated



synaptic plasticity, improved cognitive performance in the Morris water maze.³³ Besides, HRS could improve the mechanical threshold of neuropathic pain in a rat model of chronic constriction injury, which possibly by reducing oxidative stress and the subsequent expression of p38 MAPK and brain-derived neurotrophic factor.³⁴ In a controlled cortical impact model, HRW totally blocked the elevations of phosphorylated tau both in hippocampal and cortical regions, thus protected against neurodegenerative changes.³⁵ Moreover, it has been further clarified that HRW treatment decreased pro-inflammatory cytokine levels and increased anti-inflammatory cytokine levels using the same model.³⁶ In 2015, another research using a middle cerebral artery occlusion model showed that HRW treatment could not only reduce brain infarct volume and improve neurological function following ischemic brain injury, but also prevent the reduction of calcium buffering proteins, which were involved in neuronal differentiation, maturation and apoptosis and might contribute to the neuroprotective effect of hydrogen.³⁷

Neurotoxicity

In the year of 2013, it was reported that HRW reduced the degree of necrosis, apoptosis, and cell autophagy, improved place navigation ability and adaptive capacity in a rat model of acute carbon monoxide poisoning.³⁸ Another study suggested that HRW intake could protect rats exposing to low-levels of chlorpyrifos from neurotoxicity, and the protective effects of hydrogen might be mediated by regulating the oxidant and antioxidant status of rats.³⁹

The therapeutic effect of hydrogen on digestive system diseases

Gastric diseases

A report found HRW pretreatment could alleviate the aspirin-induced gastric injury by inhibiting the oxidative stress, inflammatory reaction and reducing the cyclooxygenase-2 in the gastric tissues.⁴⁰ Another study reported hydrogen-rich alkaline water exhibited a high concentration correlation with inhibitory effects showed by erosion area, myeloperoxidase (MPO) activity and malondialdehyde content in the stomach induced by aspirin-HCl, which corrected the error that the therapeutic effect of hydrogen-rich alkaline water may result from the alkaline neutralization against gastric acid.⁴¹

Liver diseases

HRW resulted in a remarkable decrease in the oxidative stress and inflammatory response, a distinct promotion of the liver function and the regeneration of liver cells in acetaminophen-induced liver injury model.⁷ Besides, a

recent report concluded HRS had significant therapeutic effects on non-alcoholic fatty liver disease induced by hyperglycemia and hyperlipidemia, which showed up as decreased expression of inflammatory factors and ROS, and increased peroxisome proliferator activated receptor α and γ levels in hepatocytes.⁴²

Pancreatic diseases

HRS also significantly attenuated the severity of acute pancreatitis (AP) *via* inhibiting the activation of NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome, which was paralleled with the decreased oxidative stress and inflammatory cascades.⁴³ A recent study further uncovered the molecular mechanism of hydrogen in the treatment of AP by proteomic analysis, namely hydrogen ameliorated the inflammatory response and reduced the expression of inflammatory mediators during the early phase of AP by inhibiting the mitogen activated protein kinases (MAPK) pathways and increasing heat shock protein 70 expression.⁴⁴

Intestinal diseases

In a neonatal rat model of necrotizing enterocolitis, HRS treatment inhibited the mRNA expression of pro-inflammatory mediators, enhanced total antioxidant capacity and prevented the increase of diamine oxidase in serum.⁴⁵ In addition, HRW was able to attenuate intussusceptions-induced intestinal I/R injury *via* inhibiting intestinal inflammation, attenuating intestinal/serum oxidative stress and reducing the number of intestinal apoptotic cells.⁴⁶ Moreover, HRW was able to enhance the anticancer activity of 5-fluorouracil by promoting cell apoptosis in colon 26 cells both *in vivo* and *in vitro*, and these effects were related to the hydrogen concentration.⁴⁷

The therapeutic effect of hydrogen on respiratory system diseases

It was widely believed that congenital diseases were irreversible for the most part, but in a recent study, Murematsu et al.⁴⁸ demonstrated the beneficial effect of hydrogen using a rat model of bronchopulmonary dysplasia by injecting lipopolysaccharide into the amniotic fluid, they found hydrogen attenuated the levels of inflammatory cytokines and ROS, increased expressions of genes for fibroblast growth factor receptor-4, vascular endothelial growth factor receptor-2, and heme oxygenase-1, thus provided a promising therapeutic strategy for bronchopulmonary dysplasia. Another study showed HRS pretreatment ameliorated cigarette smoking-induced airway mucus production and airway epithelium damage in rats.⁴⁹ Serious radiation exposure could lead to lung tissue fibrosis, which would produce serious effect on



the respiratory function. Scholars from Japan found that the hydrogen alleviated cell damage, levels of oxidative stress and apoptotic markers, thus avoiding the happening of fibrosis.⁵⁰ Besides, intraperitoneal administration of HRS before reperfusion protected the lung from I/R injury, which probably by inhibiting oxidative stress and improving antioxidant enzyme activities.⁵¹

The therapeutic effect of hydrogen on circulatory system diseases

Intraperitoneal injection of hydrogen gas was a novel hydrogen administration method and could improve the prognosis of cardiopulmonary cerebral resuscitation by alleviating oxidative stress and inhibiting apoptosis in a rabbit model of cardiac arrest.⁵² A research in 2017 demonstrated that compared with hypoxic postconditioning (HpostC) alone, the combined cardioprotective effect of hydrogen and HpostC could be better brought into play, which showed up as the reduction of infarct size, the attenuation of severe arrhythmias, and the improvement of heart function.⁵³ Another study showed that hydrogen inhalation during percutaneous coronary intervention was feasible and could also promote left ventricular reverse remodeling at 6 months after ST-elevated myocardial infarction, which was the first clinical research of hydrogen on percutaneous coronary intervention surgery for myocardial infarction.⁵⁴ However, the limited subjects could not determine the therapeutic effect of this method for sure, which need to be further confirmed.

The therapeutic effect of hydrogen on immune and hematopoietic system disorders

Cytokines such as interferon- γ , tumor necrosis factor- α and interleukin-6 secreted by autologous T cells are closely related with the development of aplastic anemia, and HRS was reported to inhibit the levels of cytokines above.⁵⁵ Furthermore, the number of peripheral blood cells and body weight were significantly increased for aplastic anemia mice in the HRS treated groups, and the bone marrow microenvironment was also significantly improved.⁵⁵ In 2014, some scholars found that hydrogen could alleviate the spleen injury and dysfunction, avoid the damage of lymphocyte and white blood cells, and promote the proliferation function recovery of blood cell caused by radiation.⁵⁶ In addition, HRW could also alleviate total body irradiation-induced hematopoietic stem cells injury with respect to cell number alteration and to the self-renewal and differentiation of hematopoietic stem cells.⁵⁶ Acute graft-versus-host disease (aGVHD) was a lethal complication of hematopoietic stem cell transplantation, which accompanied with severe inflammatory response and oxidative stress, but these could be ameliorated by intraperitoneal administration of HRS.⁵⁷

This theory was original and probably of importance, because therapeutic medical gases had never been used for aGVHD previously.

The therapeutic effect of hydrogen on endocrine system diseases

In a recent research, some scientists investigated the effects of HRW on lipid and glucose metabolism in patients with either T2DM or impaired glucose tolerance (IGT).⁵⁸ The results demonstrated that HRW significantly reduced the level of serum low density lipoprotein (LDL) levels, especially the net electronegative charge of modified LDL and urinary 8-isoprostanes, which indicated that HRW may prevent or delay the development and progression of T2DM and IGT.⁵⁸ In addition, Song et al.⁵⁹ suggested that the continued consumption of HRW decreased serum LDL-cholesterol and apolipoprotein B levels, improved dyslipidemia-injured high density lipoprotein functions, and might have a beneficial role in prevention of potential metabolic syndrome.

The therapeutic effect of hydrogen on urinal system diseases

In the year of 2010, Cardinal et al.⁶⁰ found that oral HRW could prevent the progression of chronic allograft nephropathy, and reduce the local production of inflammatory markers and the activation of inflammatory signaling cascades after allotransplantation, which provided a new therapeutic strategy for chronic allograft nephropathy. Hydrogen could also attenuate severe burn-induced early acute kidney injury by inhibiting the activation of p38, c-Jun N-terminal kinase, ERK and NF- κ B, and promoting the phosphorylation of protein kinase B.⁶¹ Besides, HRW could not only decrease Ferric nitrilotriacetate-induced nephrotoxicity, but also inhibit the incidence of renal cell carcinoma.⁶² The micturition interval and micturition volume were significantly increased by oral ingestion of HRW in bladder outlet obstruction model, and HRW also reversed the increase of bladder weight, oxidative stress markers and nerve growth factor, and thus effectively improved the bladder dysfunction induced by bladder outlet obstruction.⁶³

The therapeutic effect of hydrogen on the genital system

Hydrogen inhalation reduced the size of the endometrial explants, inhibited cell proliferation, improved superoxide dismutase, glutathione peroxidase and catalase activities, and regulated the expression of matrix metalloproteinase-9 and cyclooxygenase-2 in a endometriosis rat model.⁶⁴ Another research also reported that HRS could protect ovarian function and attenuate ovarian oxidative injury induced by cisplatin.⁶⁵ HRS could improve testis weight, testis dimen-



sions, sperm count, sperm motility and serum testosterone levels of rats exposed to the irradiation.⁶⁶

The therapeutic effect of hydrogen on motor system diseases

Subarachnoid cavity injection of HRS improved motor function in rat hindlimb, decreased cell death, inflammatory reaction and oxidative damage in a traumatic spinal cord injury model.¹⁷ HRS also significantly decreased the levels of lipid peroxide, vascular endothelial growth factor, thrombomodulin, and increased the glutathione level and microvessel density in a rabbit model of steroid-associated necrosis of the femoral head, which indicated that hydrogen might provide an alternative treatment for steroid-associated necrosis of the femoral head.⁶⁷ Researchers also investigated the beneficial effect of hydrogen in a duchenne muscular dystrophy animal model, and found hydrogen increased the spontaneous running distance and the weight of the offsprings, decreased plasma creatine kinase activities and the number of central nuclei of muscle fibers at ages 10 and 24 weeks.⁶⁸ Additionally, hydrogen tended to increase the total antioxidant and anti-apoptotic capacity in skeletal muscle at age 10 weeks.⁶⁸

Hydrogen and dermatosis

Radiation therapy could produce serious side effects on the skin, such as erythema, hair loss, desquamation, pigmentation and other symptoms.⁶⁹ A study from Japan demonstrated that inhalation of hydrogen-containing gas significantly reduced the severity of radio-dermatitis and accelerated the wound repair.⁷⁰ Meanwhile, the same effect could also be achieved by injecting HRS into the abdominal cavity, which was comparatively easier and safer than the direct application of hydrogen gas.⁷¹ In addition, HRW reduced the oxidative stress and inflammatory response, and also suppressed the production of immunoglobulin E in NC/Nga mice with atopic dermatitis.^{72,73}

Hydrogen and mental diseases

A number of experimental studies found that ROS are involved in morphine treatment or withdrawal.⁷⁴ Thus we have enough reason to believe the effect of hydrogen in ameliorating withdrawal symptoms induced by morphine. In fact, HRS significantly reduces body weight loss, jumping behavior and wet-dog shakes in mice undergoing naloxone-precipitated withdrawal, and attenuates anxiety-like behaviors in the elevated plus-maze and light/dark box tests after naloxone-precipitated withdrawal or a 2-day spontaneous withdrawal period.⁷⁵ A recent study suggested that HRW may also be helpful in alleviating depressive-like behavior by suppressing inflammasome activation, and reducing

the production of interleukin-1 β and ROS, which was the first report that hydrogen exerts its therapeutic effect on depressive disorder.⁷⁶

The therapeutic effect of hydrogen on multiple organ injuries

A recent research found that inhalation of hydrogen (2%) or hyperoxia (98%) alone improved the 14-day survival rate of zymosan-challenged mice from 20% to 70% or 60%, respectively. However, combination therapy with hydrogen and hyperoxia could increase the 14-day survival rate to 100%. Combination therapy alleviated the oxidative stress and inflammatory response of zymosan-challenged mice, and showed a more marked beneficial effect on other related indicators, such as MPO activity and lung wet/dry weight ratio, protein level of bronchoalveolar lavage fluid, serum biochemical indexes (aspartate transaminase, alanine transaminase, blood urea nitrogen and creatinine), lung, liver and kidney histopathological scores. Therefore, combination therapy with hydrogen and hyperoxia had an enhanced effect on the clinical treatment of sepsis.⁷⁷ In 2016, researchers investigated the effects of hydrogen on the alterations induced by low-dose long-term radiation by the determination of the body mass observation, forced swim test, the open field test, the chromosome aberration, the peripheral blood cells parameters analysis, the sperm abnormality, the lymphocyte transformation test, and the histopathological studies, and found that hydrogen could diminish the detriment induced by low-dose long-term radiation.⁷⁸

FUTURE DIRECTIONS

It is well known that oxidative stress is the main cause of many common diseases, and hydrogen may have several potential advantages over other free radicals scavenging agents: (i) hydrogen is permeable to cell membrane and can target organelles including mitochondria and nuclei, which is the primary site of ROS generation⁷⁹; (ii) hydrogen specially exclusively quenches detrimental ROS, such as \bullet OH and peroxynitrite (ONOO \bullet), while maintaining the metabolic oxidation-reduction reaction and other less potent ROS, such as oxygen (O₂), hydrogen peroxide (H₂O₂), and nitric oxide (NO)^{2,80}; (iii) hydrogen is obtained easily and can be oxidized into water which is safe to our body. There were some questions, also, whether these concentrations of hydrogen gas could compete effectively with the numerous cellular targets of \bullet OH, such as membrane lipids and thiols, which were in far greater abundance than hydrogen. Secondly, the published rate constant for the reaction of \bullet OH with hydrogen (H₂) to form H₂O was drastically slower than most radical-radical reactions, thus the pharmacokinetics of hydrogen remains incompletely understood.



On the other hand, the bioactivities of hydrogen were also unclear. Matsumoto et al.³⁰ recently showed that oral HRW stimulated ghrelin secretion from the stomach and protected nigrostriatal neurons from neurotoxins in PD model rats, which indicated that HRW may induce secreted factors such as ghrelin in the stomach in a dose dependent manner, where hydrogen concentration is extremely high. A recent study reported that administration of both hydrogen-containing air (HCA) and HRW had a stronger effect than administration of either alone on gene expression.¹⁰ Another similar research showed HRW, but not HCA, was effective in a PD rat model; however, by inducing a blood concentration of hydrogen similar to that produced by HRW, intermittent administration of HCA resulted in intermediate effects between HRW and the normal HCA treatment.⁸¹ According to these studies, hydrogen bioactivity depends not only on its blood concentration but also on changes in the hydrogen concentration through an unknown mechanism, which could be explained by additive effects caused by HCA and secreted factors induced by HRW.

In fact, many studies have confirmed the beneficial effects of hydrogen, and also revealed various effectors of hydrogen, but the real action mechanisms of hydrogen were still unclear. In my view, hydrogen was unlikely to directly interact with certain effectors, but could play its role indirectly. Therefore, it was not our top priority finding out the effector molecules of hydrogen. Oppositely, scientists should focus their attention on negating or excluding the molecules which were supposed as target molecules of hydrogen. Only in this way, could we find the direct targets of hydrogen and uncover the real action mechanisms of hydrogen.

CONCLUSION

All of these studies state hydrogen, as a kind of selective antioxidant, a signal pathway and gene expression modulating molecular, has very broad application prospect, which overthrew the traditional view that hydrogen is a physiological inert gas. We are looking forward to that the exact mechanism of hydrogen could be found, and more randomized and placebo controlled clinical trials will be needed to examine the therapeutic effect of hydrogen on humans.

Author contributions

HML reviewed relative articles and drafted the manuscript. JWG revised the manuscript. LS guided HML and provided original creative ideals. RFZ gave some important advices after reading the draft. All the authors read and approved the final version of the manuscript.

Conflicts of interest

No conflict of interest exists in the submission of this manuscript.

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