

The Molecular Biological Mechanism of Hydrogen Therapy and Its Application in Spinal Cord Injury

Quan Hu^{1,*}, Yingxiao Li^{2,*}, Zhaochen Lin³, Hao Zhang⁴, Haoyue Chen⁴, Cui Chao³, Chuanliang Zhao⁵

¹Department of Neurosurgery, The Affiliated Taian City Central Hospital of Qingdao University, Tai'an City, Shandong, 271000, People's Republic of China; ²Department of Gynecology, The Affiliated Taian City Central Hospital of Qingdao University, Tai'an City, Shandong, 271000, People's Republic of China; ³Hydrogen Medical Research Center, The Affiliated Taian City Central Hospital of Qingdao University, Tai'an City, Shandong, 271000, People's Republic of China; ⁴Department of Rehabilitation Medical Center, The Affiliated Taian City Central Hospital of Qingdao University, Tai'an City, Shandong, 271000, People's Republic of China; ⁵Department of Orthopedics, the Affiliated Taian City Central Hospital of Qingdao University, Tai'an City, Shandong, 271000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Chuanliang Zhao, Department of Orthopedics, The Affiliated Taian City Central Hospital of Qingdao University, Tai'an City, Shandong, 271000, People's Republic of China, Tel +8615621539631, Email zhaochuanliang2003@163.com

Abstract: Hydrogen, which is a novel biomedical molecule, is currently the subject of extensive research involving animal experiments and in vitro cell experiments, and it is gradually being applied in clinical settings. Hydrogen has been proven to possess anti-inflammatory, selective antioxidant, and antiapoptotic effects, thus exhibiting considerable protective effects in various diseases. In recent years, several studies have provided preliminary evidence for the protective effects of hydrogen on spinal cord injury (SCI). This paper provides a comprehensive review of the potential molecular biology mechanisms of hydrogen therapy and its application in treating SCI, with an aim to better explore the medical value of hydrogen and provide new avenues for the adjuvant treatment of SCI.

Keywords: hydrogen, spinal cord injury, selective antioxidant, anti-inflammatory

Introduction

Hydrogen gas is abundant in the environment. Traditionally considered an inert gas, its role in living organisms has been largely overlooked. In 1975, American researchers first reported that high-pressure hydrogen gas could be used to treat squamous cell carcinoma in mice, which is possibly due to its antioxidant properties.¹ In 2007, Japanese scholars reported that the inhalation of 2% hydrogen gas could selectively scavenge hydroxyl radicals and peroxy nitrite anions, thereby improving oxidative stress damage caused by cerebral ischemia–reperfusion and reducing the cerebral infarction area in rats. This study sparked widespread interest in the medical field regarding the value of hydrogen in medical research.² Since that time, research on hydrogen therapy has gradually expanded to include diseases such as liver cancer, chronic inflammatory responses in hemodialysis, mitochondrial myopathy, metabolic syndrome, diabetes, rheumatoid arthritis, and pneumonia, thus achieving promising results.^{3–6} Hydrogen is now recognized as being the fourth signaling gas molecule after Nitric Oxide (NO), Carbon monoxide (CO), and Hydrogen sulfide (H₂S) and is considered a novel medical gas molecule.^{7,8} This article aims to provide a comprehensive review of the potential molecular and biological mechanisms of hydrogen therapy, as well as its application in treating spinal cord injury (SCI), with the objective of better understanding the medical value of hydrogen and providing new avenues for the adjunctive treatment of SCI.

SCI and Treatment Status

Pathogenesis of SCI

SCI can be divided into two stages based on pathological changes: primary and secondary stages. Primary injury signifies irreversible mechanical damage causing tissue deformation or tearing, local blood supply disorders, neuronal and glial

damage, local axonal truncation, and neuronal destruction. Secondary injury prompts immune system activation, thus leading to rapid microglial activation and the release of proinflammatory mediators such as NO, interleukin-1 beta (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6). Cytokines produced by activated inflammatory cells, along with adhesion molecules and chemokines produced by damaged endothelial cells, promote the influx of neutrophils and monocytes into the injured spinal cord. This triggers an inflammatory response cascade that exacerbates SCI, thus ultimately leading to excitotoxicity, axonal demyelination, necrosis, glial scar formation, degeneration, and cell death of damaged cells.⁹

Treatment of SCI

Currently, treatment methods for SCI are rapidly evolving, with some methods transitioning from laboratory to clinical trials.¹⁰ Research on SCI treatment primarily focuses on three aspects: the first aspect involves reducing pressure or controlling inflammation to preserve existing injuries and to prevent further secondary damage; the second aspect involves reactivating cell function through various means to promote residual axon remyelination; and the third aspect involves nerve regeneration or reconstruction. Despite the use of various methods, no single therapy can address the issue of spinal cord injury. In the last twenty years (especially in recent years), substantial progress has been made in basic and preclinical studies (including studies on the use of hydrogen for treating SCI) and clinical therapeutic studies.^{11,12} Encouragingly, several clinical trials and studies have yielded positive results and may become the basis for new conventional treatments, including cell therapy, neuromodulation and brain-computer interfaces, neurorestorative decompression or transplantation, new neurorepair drugs, hyperbaric oxygen therapy, and other approaches.¹³

Biological Mechanisms of Hydrogen Therapy (Figure 1)

Selective Antioxidant Action of Hydrogen

Free radicals are atoms, atomic groups, or molecules with unpaired electrons, such as hydroxyl radicals (\cdot HO). Reactive oxygen species (ROS) include superoxide anions, hydrogen peroxide, peroxynitrite anions, and others.⁷ Under normal conditions, these free radicals are necessary for maintaining normal cellular functions and serve as the basis of energy

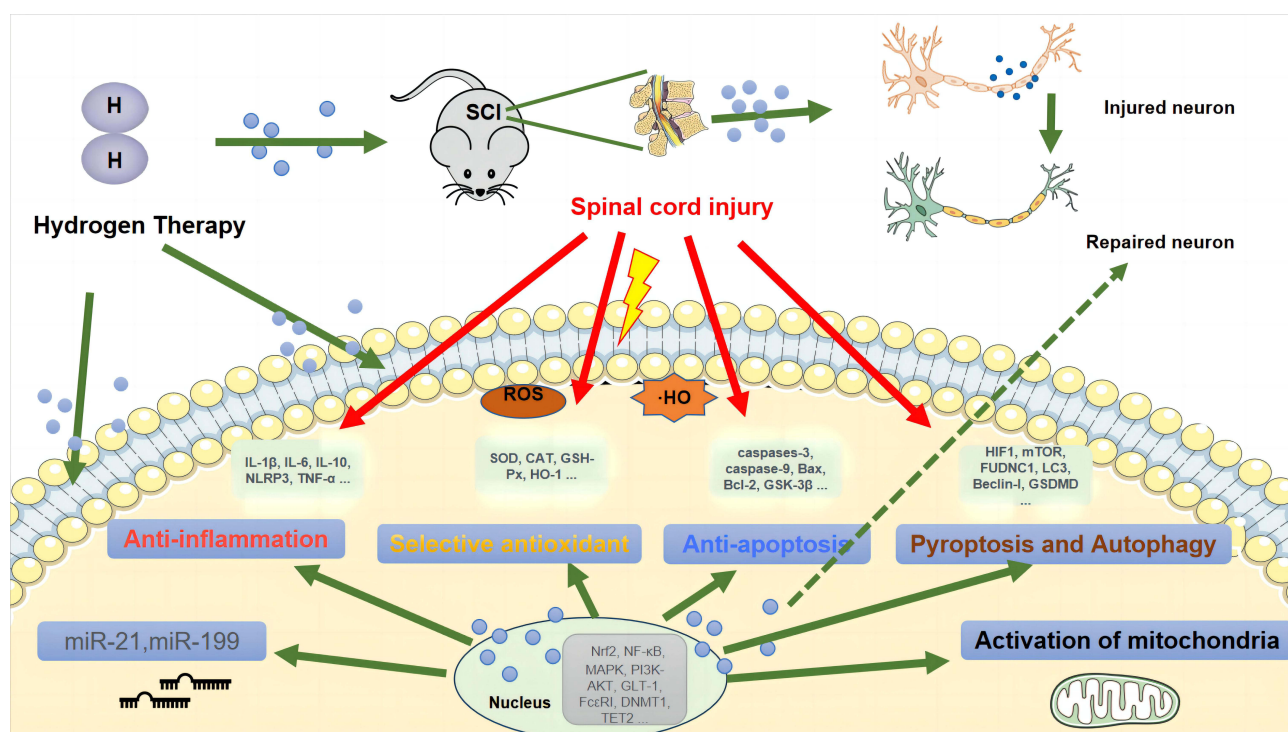


Figure 1 Molecular action mechanism of hydrogen in biology.

metabolism. Free radicals also act as important signaling molecules within cells. However, excess free radicals can cause cellular damage.¹⁴ Numerous studies have shown that hydrogen can selectively eliminate excessive harmful free radicals in the body and alleviate cellular damage.^{2,3,15–19} Du et al reported that hydrogen-rich saline can act on the ROS/NOD-like receptor thermal protein domain associated protein 3 (NLRP3) signaling pathway, alleviate inflammation, and promote recovery from cerebral ischemia.²⁰ Zhang et al reported that the inhalation of hydrogen gas significantly upregulated superoxide dismutase activity and significantly reduced the elevated levels of malondialdehyde and myeloperoxidase.²¹ In addition to directly neutralizing free radicals, hydrogen can indirectly act on the antioxidant system by enhancing the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and heme oxygenase-1 (HO-1), thereby eliminating harmful free radicals in the body and protecting against oxidative stress-induced damage.^{22–24}

Anti-Inflammatory Effects

Inflammation is an immune response that is initiated by white blood cells that target infections to restore tissue homeostasis. Uncontrolled inflammation can lead to tissue damage. Inflammatory reactions are important components in the pathogenesis of various diseases. SCI can cause complex and variable inflammatory reactions in the early stages of injury, which is one of the important pathological mechanisms of secondary SCI.²⁵ Previous studies have shown that reducing this type of inflammation can be beneficial for alleviating SCI.²⁶ Hydrogen molecules are nontoxic and efficient antioxidants that can reduce the generation of inflammatory factors and increase the release of anti-inflammatory factors.²⁷ The anti-inflammatory effects of hydrogen gas involve the expression of proinflammatory cytokines, such as IL-1 β , IL-6, interleukin-10 (IL-10), and TNF- α , as well as inflammatory mediators, such as macrophage chemoattractant protein 1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), NLRP3, high-mobility group box protein 1 (HMGB-1), and nuclear factor- κ B (NF- κ B); these factors are regulated by the Toll-like receptor 4 (TLR-4) signaling pathway, thereby exerting anti-inflammatory effects.^{28–32} Zhao et al reported that hydrogen gas alleviates inflammation by inducing the early polarization of M2 macrophages during skin wound healing.³³ Zhang et al reported that the anti-inflammatory effect of hydrogen gas is reflected not only in inhibiting the activity of inflammatory cells and reducing inflammatory cell infiltration but also in reducing important factors involved in the inflammatory response, such as 3-nitrotyrosine (3-NT), the expression of ICAM-1, and myeloperoxidase (MPO).³⁴ Yao et al demonstrated that hydrogen-rich saline improved the inflammatory response during ischemia/reperfusion by inducing mitochondrial autophagy mediated by PINK1/Parkin.³⁵ Moreover, Guo et al showed that hydrogen gas can alleviate early acute kidney injury in rats after severe burns by inhibiting the activation of p38, c-Jun amino acid terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and NF- κ B, as well as by increasing the phosphorylation of protein kinase B (Akt) and regulating the mitogen-activated protein kinase (MAPK), Akt, and NF- κ B signaling pathways, thereby suppressing the oxidative stress-induced inflammatory response.³⁶ Wang et al reported that hydrogen-rich saline alleviates the inflammatory response induced by amyloid- β in a rat model of Alzheimer's disease by inhibiting the activation of JNK and NF- κ B.³⁷

Mitochondrial Function Recovery and Reconstruction

The production of reactive oxygen species mainly occurs in the mitochondria, and research has reported that hydrogen may activate mitochondria to restore depleted CD8(+) T cells to active CD8(+) T cells, thereby improving the prognosis of patients with cancer.³⁸ Hydrogen can promote cell protection, mitochondrial biogenesis, adenosine triphosphate (ATP) generation, an increased NAD(+)/NADH ratio, Phase II enzymes for cell protection, heat shock proteins, and sirtuins, among other effects.³⁹ Dohi et al reported that treatment with hydrogen-rich culture medium for 24 hours significantly enhanced mitochondrial basal respiration, reserve respiration, and nonmitochondrial respiration while increasing ATP production.⁴⁰ Nonmitochondrial respiration refers to the oxygen consumed by enzymes in the cell after the mitochondrial electron transfer chain is closed. The authors believe that hydrogen treatment may form a hydrogen ion gradient that promotes ATP generation independent of the mitochondrial electron transport chain, thereby alleviating the decrease in ATP generation caused by mitochondrial damage due to traumatic brain injury. Yao et al showed that hydrogen-rich saline improved the inflammatory response and apoptosis in ischemia/reperfusion injury via PINK1/Parkin-mediated mitochondrial autophagy.³⁵

Regulated Cell Death

Anti-Apoptosis

Apoptosis is a highly regulated process that occurs during the normal turnover of healthy cells, as well as in various diseases, such as inflammation, infection, autoimmunity, and cancer. Apoptotic cells undergo a series of morphological changes that lead to the disintegration of dead cells. Apoptosis after secondary SCI leads to irreversible changes in neurons and exacerbates injury. Several studies have shown that hydrogen promotes functional recovery after nerve injury via its antiapoptotic effects.^{41,42} Hydrogen molecules can cross the cell membrane and enter the cell, protect DeoxyriboNucleic Acid (DNA) and proteins from damage by free radicals, maintain normal mitochondrial function, and prevent apoptosis.¹⁹ In contrast, it inhibits the expression and translocation of a variety of apoptotic proteins and upregulates the expression of antiapoptotic proteins. Additionally, hydrogen reduces the expression of caspase-3, caspase-9, and BCL2-associated X (Bax), increases the expression of B-cell lymphoma-2 (Bcl-2), and significantly reduces neuronal apoptosis.^{43,44} Hydrogen can reduce acute lung injury in septic rats, and this mechanism may be related to the regulation of macrophage polarization and a reduction in apoptosis.⁴⁵ Furthermore, hydrogen protects the intestinal epithelial tight junction barrier by inhibiting the endoplasmic reticulum stress-induced apoptotic pathway in rats with intestinal ischemia–reperfusion injury.⁴⁶ Hydrogen can also promote apoptosis and autophagy in lung cancer cells by inhibiting the activation of the STAT3/Bcl2 signaling pathway, and the inhibition of autophagy can enhance the ability of hydrogen to promote the apoptosis of lung cancer cells.⁴⁷ Hydrogen treatment of scratch-injured cells can inhibit cell apoptosis, mainly by activating the PI3K/Akt/GSK-3 β signaling pathway.⁴⁸ The antiapoptotic effect of hydrogen primarily occurs by inhibiting the expression and translocation of apoptotic proteins and upregulating the expression of antiapoptotic proteins.

Autophagy Suppression

Recent studies have shown that hydrogen may exert a protective effect by regulating the autophagy signaling pathway. In a rat model of neuropathic pain, Wang et al reported that hydrogen-rich saline alleviated mechanical hyperalgesia and autophagy activation through an HIF1-dependent pathway.⁴⁹ Zhu et al reported that hydrogen regulates the polarization of microglia through the mechanistic target of rapamycin (mTOR) autophagy-dependent pathway, thus reducing sepsis-induced neuroinflammation. According to animal and cellular studies, hydrogen reduces TNF- α , IL-6, and HMGB-1 levels and M1-type polarization but increases IL-10 and transforming growth factor- β (TGF- β) horizontal and M2-type polarization. Hydrogen treatment reduces the P-mTOR/mTOR ratio and the expression of P62 while increasing the phosphorylated adenosine 5'-monophosphate-activated protein kinase (AMPK)/AMPK ratio, as well as the ratio of LC3II/LC3I, the recombinant triggering receptor expressed on myeloid cells 2 (TREM-2), and Beclin-1 expression.⁵⁰ Liu et al demonstrated that hydrogen can promote apoptosis and autophagy in lung cancer cells by inhibiting the activation of the STAT3/Bcl2 signaling pathway and that inhibiting autophagy can enhance the ability of hydrogen to promote apoptosis in lung cancer cells.⁴⁷ Gao et al reported that hydrogen alleviates myocardial ischemia–reperfusion injury in rats by weakening endoplasmic reticulum stress-induced autophagy.⁵¹ Yan et al reported that the therapeutic effect of hydrogen on sepsis-induced liver injury may be mediated by the regulation of the mitochondrial autophagy pathway induced by Fun14 domain protein 1.¹⁹ In a rat model of postherpetic neuralgia, a hydrogen-rich solution activated autophagy by upregulating the expression of LC3, Beclin-I, and p62, thus reducing postherpetic neuralgia in rats.⁵²

Antipyroptosis

Mu et al reported that hydrogen produced by hydrogen-producing silicones may regulate microglial pyroptosis through the NLRP3-caspase-1-GSDMD pathway, thereby preventing the development of chronic neuroinflammation and reducing the incidence of nerve demyelination.⁵³ Luo et al reported that hydrogen may alleviate acute lung injury after burn injury by inhibiting macrophage apoptosis via NLRP3.⁵⁴ Zou et al reported that hydrogen reduced cell apoptosis by inhibiting the AMPK/mTOR/NLRP3 signaling pathway.⁵⁵ Additionally, the hydrogen storage nanomaterial MgH(2) inhibited cell apoptosis, pyroptosis, and inflammatory responses by mediating irradiation-induced ROS production, thereby improving radiation-induced damage to male fertility.⁵⁶ Zhang et al examined the effect of hydrogen gas on early neuronal apoptosis in rats with subarachnoid hemorrhage through the mitoK(ATP)/ERK1/2/p38 MAPK signaling pathway.⁵⁷

Methylation

Hydrogen can reduce the expression of proinflammatory factors in septic mice and decrease the expression of DNA methyltransferase 1 (DNMT1) and DNMT3a, thus leading to hypomethylation of the five CpG sites of the BDNF promoter IV. This action reduces neuroinflammation and cognitive dysfunction in septic mice.⁵⁸ In a clinical study involving older individuals (aged 70 years and above), a 6-month intake of hydrogen-rich water significantly increased DNA methylation markers compared to those in the control group. Moreover, the interaction between treatment and time exhibited a strong trend in terms of methylation degree.⁵⁹

MicroRNAs (miRNAs)

SCI leads to the abnormal expression of spinal cord miRNAs, which play a role in the pathophysiology of SCI. Hydrogen gas can promote the recovery of spinal cord function by regulating miRNAs.⁶⁰ MicroRNA-21 (miR-21) is among the noncoding small molecule miRNA family members with the highest expression level in mammalian cells. Studies have indicated that hydrogen therapy can enhance neurological prognosis following traumatic brain injury by increasing miR-21.⁶¹ Additionally, research has demonstrated that hydrogen can downregulate miR-9 and miR-21 while upregulating miR-199, thereby mitigating inflammatory damage.^{7,62} Hydrogen-saturated physiological saline significantly reduces miR-199a-3p, thereby exerting antiapoptotic effects through the IGF-1/PI3K/Akt/mTOR pathway and protecting the intestinal tract of mice from ischemia/reperfusion injury.⁶³

Biomarkers and Pathways

Hydrogen and Nuclear Factor E2 Related Factor 2 (Nrf2)

Nrf2 is a key regulatory factor in the endogenous antioxidant defense system of cells and controls the expression of antioxidant and electrophilic stress genes. It also serves as a pivotal transcription factor maintaining cellular metabolism, redox balance, and protein homeostasis, thus inducing the expression of numerous protective and detoxification genes.⁶⁴ Multiple studies have demonstrated that hydrogen exerts its antioxidant effects by activating the Nrf2 pathway.^{65–68} Kawamura et al found⁶⁷ that inhaling 2% hydrogen could upregulate the transcription of Nrf2-dependent cell protective genes, thus increasing HO-1 protein expression and mRNA transcription and thereby improving lung permeability, pulmonary edema, and oxygenation. In rats with Nrf2 gene deletion, hydrogen can still reduce malonaldehyde (MDA) levels; however, it fails to upregulate the expression of HO-1 and other Nrf2-dependent protective genes or significantly enhance body oxygenation. Hydrogen not only directly clears $\cdot\text{HO}$ but also protects body tissues and cells through the Nrf2 signaling pathway. Sun et al⁶⁵ showed that high concentrations of hydrogen can alleviate lipopolysaccharide-induced lung injury in mice by activating the Nrf2/NF- κB signaling pathway. Yu et al found²⁴ that hydrogen reduces the release of HMGB-1 in the lung tissue of septic mice through the Nrf2/HO-1 pathway. Increasing evidence suggests that the transcription factor Nrf2 crucially controls the expression of antioxidant genes, thus exerting anti-inflammatory effects. Activation of the Nrf2 pathway may play dual roles in both antioxidant and anti-inflammatory effects.⁶⁹ The antioxidant effect of hydrogen is widely acknowledged in the academic community, with oxidative stress considered to be a substantial contributor to SCI.^{70,71} Therefore, the antioxidant theory appears to elucidate the protective effects of hydrogen.

Hydrogen and NF- κB

NF- κB is a rapid-acting transcription factor that is commonly found in the cytoplasm, and it is primarily involved in transmitting information related to the body's defense response, tissue damage and stress, cell differentiation and apoptosis, as well as for inhibiting tumor growth processes. Research has demonstrated that inhaling hydrogen can reduce oxidative stress and the inflammatory response to alleviate liver damage through the TLR-4/NF- κB pathway while also enhancing the intestinal microbiota and fortifying the intestinal barrier.³⁰ An et al discovered that estrogen increased the sensitivity of male mice with cerebral hemorrhage to hydrogen gas. This mechanism may be linked to the inhibition of the astrocyte NF- κB signaling pathway.⁷² Huang et al reported that inhaling hydrogen gas significantly inhibited NF- κB and activated the Nrf2 pathway.⁷³ Moreover, inhalation of hydrogen can inhibit the ERK1/2 and NF- κB pathways,

thus reducing inflammation induced by cigarette smoke in mice with chronic obstructive pulmonary disease and impeding the progression of chronic obstructive pulmonary disease.⁷⁴ Shi et al reported that hydrogen inhibits the NF- κ B signaling pathway and the release of proinflammatory cytokines, thus mitigating sevoflurane neurotoxicity in neonatal rats.⁷⁵

Hydrogen- and Mitogen-Activated Protein Kinase (MAPK)

The MAPK pathway is a crucial signal transduction system that mediates cellular responses; moreover, it is widely present in mammalian cells and is involved in various physiological processes, such as cell growth, development, differentiation, and apoptosis. MAPKs belong to the serine/threonine kinase family and include ERKs, p38 mitogen-activated protein kinases, and JNKs, which serve as vital links in a series of kinase enzymatic cascade reactions. You et al reported that hydrogen inhibits oxidative stress-induced MAPK activation and maintains calcium homeostasis.¹⁸ Song et al reported that hydrogen inhalation alleviated cognitive dysfunction, behavioral abnormalities, and neuronal apoptosis in subarachnoid rats. This mechanism may be related to inhibition of the p38 MAPK signaling pathway.⁷⁶ The protective effects of hydrogen on organs and cells are closely associated with the activation of MAPKs and their signaling pathways.

Hydrogen and PI3K-AKT

The PI3K/Akt pathway is a notable signaling pathway that activates downstream extracellular signals and influences processes such as cell proliferation, apoptosis, and survival.⁷⁷ Ke et al reported that hydrogen alleviates neurobehavioral deficits and cell apoptosis due to hypoxic–ischemic brain injury in newborn mice via the Akt pathway. These effects, which are associated with reduced phagocytosis, seem to involve the Akt signaling pathway.⁷⁸ Hydrogen-saturated physiological saline can notably reduce miR-199a-3p, thereby exerting antiapoptotic effects through the IGF-1/PI3K/Akt/mTOR pathway to protect the intestinal tract of mice from ischemia/reperfusion injury.⁶³ Chen et al demonstrated that hydrogen-rich saline alleviated brain injury and inhibited microvascular endothelial cell apoptosis in rats undergoing cardiopulmonary bypass through the PI3K/Akt/gsk3 β signaling pathway.⁷⁹

Hydrogen and IgE High-Affinity Receptors (Fc ϵ RI)

The specific receptor Fc ϵ RI is closely associated with allergic reactions in the body. Fc ϵ RI triggers downstream signal transduction, thus leading to mast cell degranulation and the release of chemokines and cytokines. Research indicates that hydrogen can inhibit mast cell degranulation and the release of inflammatory mediators such as leukotrienes, which are initiated by IgE binding to Fc ϵ RI. This inhibition is achieved by reducing the phosphorylation of downstream molecules, including spleen tyrosine kinase (Syk), phospholipase C gamma 1 (PLC γ 1), phospholipase C gamma 2 (PLC γ 2), Akt, ERK1/2, JNK, p38, and cytosolic phospholipase A2 (cP-LA2), among other factors, in the Fc ϵ RI signaling pathway. Additionally, hydrogen reduces NADPH oxidase activity, which subsequently decreases H₂O₂ production. There is a close relationship between hydrogen and Fc ϵ RI, thus suggesting that hydrogen exerts protective effects through the Fc ϵ RI signaling pathway.⁸⁰

Basic and Preclinical Studies of Hydrogen Therapy for Diseases

Due to their small molecular size,⁸¹ hydrogen molecules possess high penetrability, thus enabling direct crossing of the blood–brain barrier and cell membranes without relying on protein channels. Consequently, they diffuse into the spinal cord, systemic tissues, and organs, where they exert biological effects.^{7,19} Hydrogen, which is a weak reducing agent, does not react with reactive oxygen species, which are crucial in signaling. Instead, it selectively scavenges free oxygen radicals, thus preserving other beneficial oxidants and the body's inherent antioxidant defense system.⁸² Moreover, hydrogen gas functions as an inert molecule within the human body and is unaffected by signaling molecules involved in neural transmission, vasodilation, cell apoptosis, proliferation, differentiation, extensive signal cascades, and anti-infectious signals (such as NO and O₂· H₂O₂)^{83,84} (Figure 2). Many basic and clinical studies have explored the role of hydrogen in various conditions, including respiratory diseases,^{27,81,85–88} cardiovascular diseases,⁸⁹ neurological disorders,^{90,91} gastrointestinal diseases,^{92,93} musculoskeletal disorders,^{94,95} immune system disorders,⁹⁶ injury repair,⁹⁷

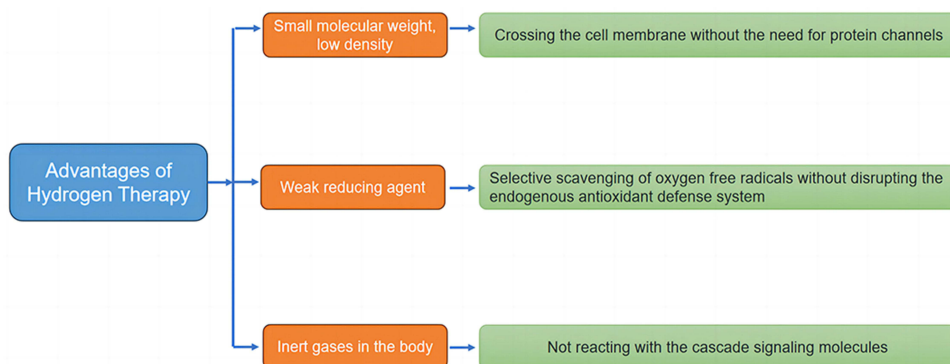


Figure 2 Advantages of hydrogen therapy in treating spinal cord injury.

cancer,⁹⁸ and multiorgan damage caused by COVID-19.^{88,99,100} Overall, hydrogen therapy has shown potential in treating numerous diseases.

Currently, various approaches exist for delivering hydrogen in SCI treatment (Figure 3), including intraperitoneal injections of hydrogen-rich saline,¹⁰¹ subarachnoid injections of hydrogen-rich saline,⁴⁴ inhalation of hydrogen gas,⁴³ and slow release of hydrogen using novel materials.¹⁰² Studies on SCI treatment using hydrogen have included three cases involving hydrogen gas inhalation,^{43,103,104} one case involving subarachnoid injection,⁴⁴ and one case involving the use of a novel material (anisotropic magnetic fluid hydrogel).¹⁰² Other studies have used intraperitoneal injections of hydrogen-rich physiological saline. Hydrogen-rich physiological saline is commonly used due to its perceived safety, ease of administration, ability to enter the bloodstream, and ability to facilitate hydrogen transportation and diffusion within the body with precise dosage calculations. However, its use is limited due to its relatively low solubility in hydrogen gas at normal temperatures and pressures, thus leading to potential hydrogen leakage. Hence, a newly prepared hydrogen-rich physiological saline with improved sealing ability is preferred. There are no standardized guidelines for hydrogen dosage. Most studies have administered the dosage once daily throughout the experiments, often at 5 mL/kg for intraperitoneal injections. A greater amount of hydrogen-rich water had a more significant therapeutic effect. Kimura et al compared inhalation dosages of 1%, 2%, and 3% hydrogen gas, and they observed better treatment results with a 3% dosage.¹⁰³ Chen et al compared inhalation dosages of 50%, 65%, and 75% hydrogen gas, with the 75% dosage showing superior treatment effects.⁴³ Similarly, higher doses of hydrogen-rich physiological saline solutions resulted in better treatment effects.^{105,106} From the perspective of existing studies and pharmacology, it is easier to quickly obtain a higher dose of hydrogen intake by using a relatively high concentration of hydrogen, which is more likely to produce possible

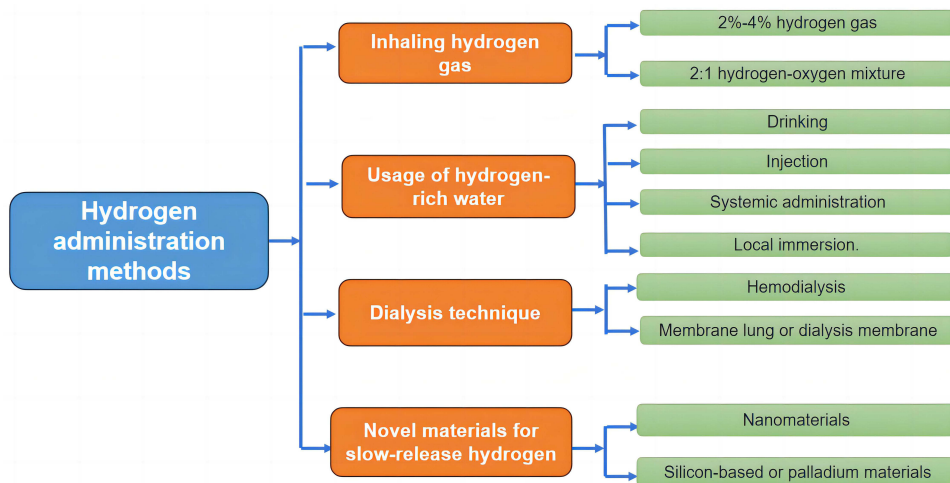


Figure 3 Methods of hydrogen delivery.

effects.^{107–110} However, blindly pursuing high concentrations may cause unnecessary treatment risks. At present, research on hydrogen medicine lacks a strict dose-effect relationship, which may be one of the key research directions of hydrogen medicine in the future. Currently, other methods of hydrogen delivery exist, including hydrogen delivery through hemodialysis and peritoneal techniques,³ topical application,¹¹¹ and subcutaneous injections,¹¹² among other methods. The emergence of novel hydrogen-producing materials may further enhance the efficacy of existing intervention methods. They enable slow hydrogen release and targeted applications, thus potentially making hydrogen therapies safer and more effective.¹⁰²

Research Status of Hydrogen Therapy for Spinal Cord Injury

Since 2010, 12 studies on the protective effect of hydrogen on SCI have been published (Table 1), which have preliminarily confirmed the potential protective effect of hydrogen on SCI.

These studies have confirmed (from multiple perspectives) that hydrogen therapy can reduce the oxidative stress response after SCI, alleviate inflammation, prevent apoptosis, improve spinal cord ischemia–reperfusion injury, inhibit astrocyte formation, inhibit chemokines, and restore mitochondrial function (Figure 4).

Selective Antioxidant, Anti-Inflammatory and Antiapoptotic Effects

In 2010, Chen et al examined the mechanism of action of hydrogen-rich saline in treating acute spinal cord contusions. The authors used a standard contusion SCI model with male Sprague–Dawley rats. Hydrogen saline (5 mL/kg) was

Table 1 Basic Research of Hydrogen in the Treatment of Spinal Cord Injury

Cells/Animals	Treatments	Duration	Effects	Biomarkers Evaluated	References
BV2 cell/Sprague–Dawley rats	Ferromagnetic fluid hydrogel for slow release of hydrogen	8 weeks post injury	Reducing the expression of activated microglia and proinflammatory factors in SCI rats through the NF- κ B pathway. Significantly promotes axonal directed regeneration and functional recovery after spinal cord injury	IL-1 β ↓, IL-6↓, iNOS↓, TNF- α ↓, P-P65↓, P-I κ B↓	[102]
Bone mesenchymal stem cell/Sprague–Dawley rats	Hydrogen-Rich Cell Culture Medium/ Hydrogen-Rich Saline	28 days post injury	Hydrogen can repair spinal cord injury by reducing inflammatory response and oxidative stress at the injury site and enhancing the migration and proliferation of bone marrow mesenchymal stem cells	TNF- α ↓, IL-1 β ↓, IL-6↓, MDA↓, SOD↑	[101]
Sprague–Dawley rats	Hydrogen-Rich Saline	7 days post injury	The levels of IL-6 and TNF- α were significantly decreased in hydrogen-rich saline treated group after SCI, and apoptosis was also significantly reduced	TNF- α ↓, IL-6↓	[113]
Sprague–Dawley rats	Hydrogen gas inhalation	140 minutes	Hydrogen inhalation exerts a concentration-dependent protective effect on the spinal cord, and GLT-1 is involved in this protective effect	GLT-1↓	[103]
Sprague–Dawley rats	Hydrogen-Rich Saline	7 days from surgery	Hydrogen-rich saline can dose-dependently decrease morphine tolerance, reduce proinflammatory cytokine expression, inhibit astrocyte activation, reduce GLT-1 and GS nitration, and inhibit membrane transport of NMDA receptors	TNF- α ↓, IL-1 β ↓, IL-6↓, GLT-1↓, GS nitration↓, NMDA receptors↓	[106]

(Continued)

Table 1 (Continued).

Cells/Animals	Treatments	Duration	Effects	Biomarkers Evaluated	References
Primary spinal cord neuron, C57BL/6 mice	Hydrogen gas inhalation	42 days post injury	Hydrogen can reduce the production of ROS and inhibit neuronal apoptosis by oxidative stress, restoring the construction and function of mitochondria in spinal cord injury. Cox8b, Cox6a2, Cox7a1, Hspb7, and Atp2a1 were identified as potential target genes for hydrogen treatment	Cox8b↓, Cox6a2↓, Cox7a1↓, Hspb7↓, Atp2a1↓	[43]
Wistar rats	Hydrogen-Rich Saline	42 days post injury	Hydrogen rich saline can promote the recovery of testicular biological function through antioxidant, anti-inflammatory, and antiapoptotic effects after spinal cord injury	HO-1↓, MFN-2↑	[114]
Sprague–Dawley rats	Hydrogen-Rich Saline	2 weeks post injury	Injecting hydrogen rich saline within 2 weeks after injury can effectively promote the repair of acute spinal cord injury, which is achieved by alleviating tissue damaging oxidative stress, inflammation and apoptosis	CGRP↑, caspase-3↓, SOD↑, MDA↓	[44]
Astrocytes/Sprague–Dawley rats	Hydrogen-Rich Saline	2 weeks post injury	Hydrogen could suppress reactive astrogliosis after contusive SCI and reduce the release of proinflammatory cytokines produced by active astrocytes related to oxidative injury	ROS↓, STAT3↓, p-STAT3↓, GFAP↓, TNF-α↓, IL-1β↓, IL-6↓,	[70]
New Zealand white rabbits	Hydrogen-Rich Saline	72 hours after reperfusion	Hydrogen therapy for spinal cord ischemia–reperfusion injury by activating the mitoKATP channel and reducing oxidative stress, inflammatory cytokines, and apoptosis	mitoKATP	[105]
Sprague–Dawley rats	Hydrogen-Rich Saline	72 hours post injury	Hydrogen-rich saline reduced acute spinal cord contusion injury, possibly by reduction of oxidative stress and elevation of BDNF	BDNF↑, MDA↓, MPO↓, caspase-3↓	[115]
New Zealand white rabbits	Hydrogen gas inhalation	72 hours after reperfusion	Hydrogen therapy is beneficial to spinal cord I/R damage by reducing oxidative stress, inflammatory response, and apoptosis	TNF-α↓, HMGB1↓, SOD↑, CAT↑, MDA↓, 8-iso-PGF2α↓	[104]

Abbreviations: Akt, Protein Kinase B; AMPK, Adenosine 5'-monophosphate-activated protein kinase; ATP, adenosine triphosphate; Bax, BCL2-Associated X; BBB, Basso, Beattie & Bresnahan locomotor rating; Bcl-2, B-cell lymphoma-2; BDNF, Brain-derived neurotrophic factor; CAT, Catalase; CO, Carbon monoxide; Cox6a2, Cytochrome c oxidase subunit 6A2; Cox7a1, Cytochrome c oxidase subunit 7A1; Cox8b, Cytochrome c oxidase subunit 8B; cP-LA2, Cytosolic Phospholipase A2; DNA, DeoxyriboNucleic Acid; DNMT, DNA methyltransferase; ERK, extracellular signal-regulated kinase; ERK, Extracellular regulated protein kinases; FcεRI, IgE High Affinity Receptors; GLT-1, Glutamate transporter-1; GS, Glutamine synthetase; GSDMD, Gasdermin D; GSH-Px, Glutathione peroxidase; GSK-3β, Glycogen synthase kinase-3 beta; HIF1, hypoxia inducible factor-1; HMGB-1, High-mobility group box protein 1; HO-1, oxygenase-1; H₂S, Hydrogen sulfide; Hspb7, Heat Shock Protein Beta 7; ICAM-1, Intercellular adhesion molecule 1; IGF-1, Insulin-like growth factor 1; IL-10, Interleukin-10; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; JNK, c-Jun amino acid terminal kinase; LC3, Microtubule-associated protein light chain 3; MAPK, Mitogen Activated Protein Kinase; MCP-1, Macrophage chemoattractant protein 1; MDA, malonaldehyde; MPO, Myeloperoxidase; mTOR, Mechanistic Target Of Rapamycin; NAD, Nicotinamide adenine dinucleotide; NF-κB, Nuclear factor-κB; NLRP3, NOD-like receptor thermal protein domain associated protein 3; NMDA, N-Methyl-d-aspartic acid; NO, Nitric Oxide; Nrf2, Nuclear factor E2 related factor 2; PI3K, Phosphatidylinositol 3-kinase; PINK1, PTEN induced putative kinase 1; PLCγ1, Phospholipase C Gamma 1; PLCγ2, Phospholipase C Gamma 2; ROS, Reactive oxygen species; SCI, Spinal cord injury; SOD, superoxide dismutase; STAT3, Signal transducer and activator of transcription; Syk, Spleen Tyrosine Kinase; TGF-β, Transforming growth factor-β; TLR-4, Toll-like receptor 4; TNF-α, Tumor necrosis factor-α; TREM-2, Recombinant Triggering Receptor Expressed On Myeloid Cells 2; HO, hydroxyl radicals; 3-NT, 3-nitrotyrosine; 8-iso-PGF2α, 8-iso-prostaglandin F2alpha.

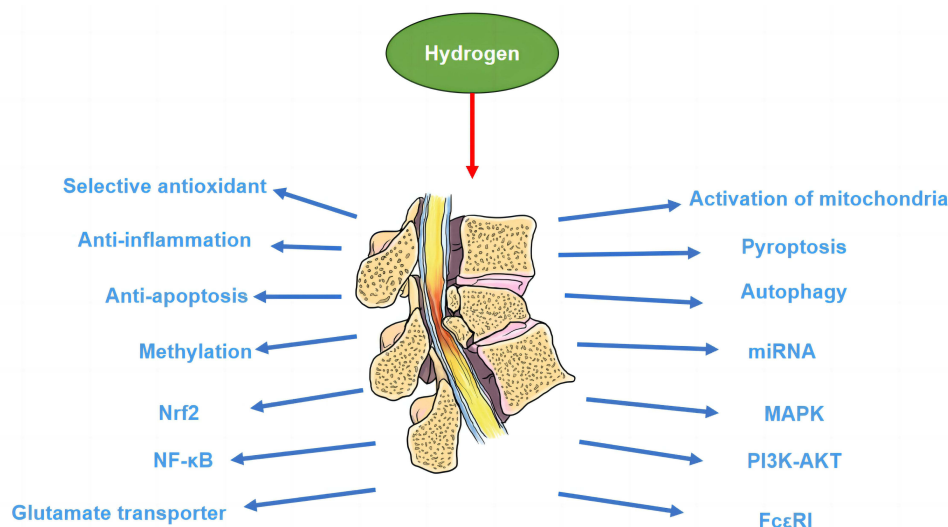


Figure 4 Protective effect of hydrogen on spinal cord injury and its possible molecular mechanism.

intraperitoneally injected at 24 and 48 hours after injury. Cell apoptosis, oxidative stress, inflammation, and brain-derived neurotrophic factor (BDNF) levels were measured. They also utilized the Basso, Beattie & Bresnahan locomotor rating (BBB) scale to evaluate exercise behavior. Research has indicated that hydrogen-rich saline may alleviate acute SCI by reducing oxidative stress, decreasing the number of apoptotic cells, and increasing BDNF levels.¹¹⁵ Huang investigated the therapeutic effect of hydrogen on spinal cord ischemia–reperfusion injury in rabbits. One study demonstrated that hydrogen reduced oxidative stress and the inflammatory response in a concentration-dependent manner.¹⁰⁴ Liu et al conducted *in vitro* and *in vivo* experiments and found that hydrogen molecules could inhibit the formation of reactive astrocytes after traumatic SCI. This inhibition led to a reduction in the release of proinflammatory cytokines produced by active astrocytes associated with oxidative damage.⁷⁰

Wang et al reported that hydrogen-rich saline notably reduced cell death, inflammatory cell infiltration, serum malondialdehyde content, and caspase-3 immunoreactivity. It also increased serum superoxide dismutase activity and calcitonin gene-related peptide immunoreactivity, thus ultimately improving hind limb motor function.⁴⁴ Ge et al demonstrated that hydrogen-rich saline could enhance the recovery of testicular function following spinal cord hemisection injury through its antioxidant, anti-inflammatory, and antiapoptotic effects.¹¹⁴ Kayabas et al investigated the anti-inflammatory and antiapoptotic effects of hydrogen-rich saline by reducing the levels of IL-6 and TNF- α .¹¹³

Activation of Mitochondria

Chen et al reported that the inhalation of 75% hydrogen gas improved SCI, reduced mechanical damage to isolated neurons, and exerted a dose-dependent protective effect on spinal cord neurons. These mechanisms include reducing ROS production and oxidative stress, inhibiting neuronal apoptosis, and restoring mitochondrial structure and function. Cytochrome c oxidase subunit 8B (Cox8b), cytochrome c oxidase subunit 6A2 (Cox6a2), cytochrome c oxidase subunit 7A1 (Cox7a1), heat shock protein beta 7 (Hspb7), and Atp2a1 have been identified as being potential targets of hydrogen treatment.⁴³ MitoKATP is a protein complex that is present in mitochondria that is associated with ATP levels. Zhou et al induced spinal cord ischemia for 20 min in male New Zealand white rabbits with subrenal aortic occlusion. Different doses of hydrogen-rich saline were intravenously administered before or 5 min after the start of reperfusion. Studies have shown that hydrogen-rich saline may be an effective therapeutic agent for spinal cord ischemia–reperfusion injury by reducing oxidative stress, inflammatory cytokines and apoptosis, and this therapeutic effect can be partially eliminated by the mitoKATP channel blocker 5-hydroxyacetate.¹⁰⁵

Targeting Glutamate Transporter-1 (GLT-1)

Glutamate is the most important excitatory neurotransmitter in the spinal cord and brain, and glutamate transporters play an extremely important role in maintaining normal extracellular glutamate concentrations and physiological activities of the central nervous system. Glutamine synthetase (GS) is involved in glutamate synthesis, and N-methyl-D-aspartic acid (NMDA) is a homolog of glutamate and an important neurotransmitter in the spinal cord. Li et al reported that hydrogen preconditioning prevents morphine tolerance by reducing neuroinflammation, GLT-1, glutamine synthetase nitration, and NMDA receptor transport in the spinal dorsal horn. Thus, hydrogen preconditioning can be used as a new therapeutic strategy to prevent morphine tolerance.¹⁰⁶ Kimura et al reported that inhaled hydrogen reduced the increase in glutamate levels in the anterior cornua of the spinal cord that was induced by spinal cord ischemia–reperfusion injury in a concentration-dependent manner and played a protective role in the injured spinal cord. Further studies have shown that the protective effect of hydrogen is eliminated by using GLT-1 inhibitors. The classical mechanism of action of hydrogen involves selective oxidation. This study identified a new molecular mechanism by which hydrogen can directly act on GLT-1 to play a therapeutic role without the use of selective antioxidants.¹⁰³

Combination with Bone Marrow Mesenchymal Stem Cells

Luo et al reported that the codelivery of hydrogen and bone marrow stem cells can reduce inflammation and oxidative stress at the injury site, improve the survival rate and migration ability of transplanted cells, significantly promote the recovery of neural function, and repair the injured spinal cord.¹⁰¹

Slow Release of Hydrogen Through New Materials

Anisotropic ferromagnetic fluid hydrogels inhibit the NF- κ B pathway by slowly releasing hydrogen, reducing the expression of activated microglia/macrophages and proinflammatory factors in SCI rats, and significantly promoting axonal directional regeneration and functional recovery after SCI.¹⁰²

Clinical Therapeutic Studies of Hydrogen Therapy for Diseases and Damage

To date, no clinical studies on hydrogen therapy for SCI have been reported; however, many clinical studies have been conducted on craniocerebral diseases. Benjamin reported that hydrogen-rich saline can be used in patients with traumatic brain injury.¹¹⁶ A study by Tamura, which screened 429 patients who experienced cranial coma after out-of-hospital cardiac arrest between February 1, 2017, and September 30, 2021, demonstrated that hydrogen inhalation may increase birth survival without neurological dysfunction by 90%.¹¹⁷ In a randomized, double-blind placebo-controlled clinical study, 73 patients with mild cognitive impairment drank approximately 300 mL of hydrogen-rich saline or placebo water per day (control group). After 1 year, apolipoprotein E4 genotype carriers in the hydrogen treatment group showed significant improvements in the total Alzheimer's Disease Assessment Scale cognitive subscale score and word recall task score.¹¹⁸ Ono et al evaluated eight patients with Alzheimer's disease who inhaled 3% H₂ gas for one hour twice daily for six months. Studies have shown that hydrogen treatment not only alleviates clinical symptoms but also significantly improves the integrity of neurons passing through the hippocampus.¹¹⁹ A total of 38 hospitalized patients with acute ischemic stroke were treated with a combination of conventional treatment and hydrogen-rich saline, and research data has shown that intravenous injection of HRS-assisted treatment for acute ischemic stroke was safe.¹²⁰

Hydrogen has been used as a diagnostic marker for several diseases. Yokoyama examined 107 patients with cirrhosis and reported that breath measurements of hydrogen and methane concentrations can be used to diagnose intestinal bacterial overgrowth and, subsequently, the occurrence of cirrhosis.¹²¹ Europe-wide diagnostic indicators for hydrogen and methane breath testing have been used for symptom assessment in the diagnosis of carbohydrate intolerance in both adult and pediatric patients and have been included in clinical guidelines.¹²² Future diagnosis of hydrogen markers based on hydrogen-producing bacteria will provide a new perspective for the diagnosis and prevention of Alzheimer's disease.¹²³ Whether hydrogen can be used as a diagnostic biomarker of SCI in the future needs further research.

Limitations of Current Research on Hydrogen Therapy

Hydrogen medicine, which was developed in 1975, has a history of nearly 50 years and has achieved significant results in basic and clinical research, as well as in various applications. However, these studies were essentially effect studies, and research on SCI is particularly limited, especially in terms of the lack of related clinical research. There is also a lack of research on contraindications for hydrogen therapy. Comparatively few studies have investigated the effects of hydrogen treatment in comparison to other therapeutic drugs. Moreover, there is a lack of guidelines or research consensus related to hydrogen. As a natural substance with no complex structure or preparation difficulty, hydrogen cannot be patented, and many scholars remain concerned about its safety while lacking the support of large pharmaceutical companies. In summary, research into the protective effects of hydrogen on SCI is still in its infancy. Addressing the aforementioned problems could be an important basis for promoting the clinical application of hydrogen.

Conclusions and Perspectives

Hydrogen, which is a novel biomedical molecular gas, has been extensively studied in animal and in vitro cell experiments, and there have been many clinical reports on its use in the treatment of various diseases. Hydrogen treatment has been described in the guidelines for the treatment of COVID-19 in China.⁹⁹ Medical insurance in China covers patients who receive hydrogen therapy. Hydrogen plays a role in the treatment of SCI through its biological effects, such as antioxidant, anti-inflammatory, and antiapoptotic effects. Despite its potential, research on the treatment of SCI with hydrogen remains relatively scarce, particularly in terms of clinical reports. The molecular mechanism of action of hydrogen therapy for SCI remains unclear, and research on the mechanism of action is insufficient. Currently, there is no consensus on the dosage and route of hydrogen gas. Hydrogen has great potential for application in SCI treatment. It is believed that ongoing research on hydrogen will hopefully improve the prognosis of patients with SCI in the future.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no competing interests in this work.

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