

Hydrogen therapy: from mechanism to cerebral diseases

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Abstract

The medicinal value of hydrogen (H₂) was ignored prior to research illustrating that inhalation of 2% H₂ can significantly decrease the damage of cerebral ischemia/reperfusion caused by oxidative stress *via* selective elimination of hydroxyl freebase (OH) and peroxy-nitrite anion (ONOO⁻). Subsequently, there have been numerous experiments on H₂. Most research and trials involving the mechanisms underlying H₂ therapy show the effects of antioxygenation, anti-inflammation, and anti-apoptosis. Among quantities of diseases related with H₂ therapy, the brain disease is a hotspot as brain tissue and cell damage are easier to be induced by oxidative stress and other stimulations. In this review, emphasis is on stroke, traumatic brain injuries, and degenerative diseases, such as Alzheimer's disease and Parkinson's disease. Taking into account the blood-brain barrier, penetrability, possible side effects, and the molecular properties of H₂ within a single comprehensive review should contribute to advancing both clinical and non-clinical research and therapies. A systematic introduction of H₂ therapy with regards to mechanisms and cerebral diseases both in animal and human subjects can make it easier to comprehend H₂ therapy and therefore provide the basis for further clinical strategy.

Key words: hydrogen therapy; ingestion; oxidative stress; inflammation; apoptosis; cerebral diseases

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INTRODUCTION

Hydrogen (H₂) therapy has been an intense subject of interest following the discovery of selective antioxygenation by Ohsawa in 2007 (Ohsawa et al., 2007). Currently, there are only a minority of antioxidants used in the clinical treatment of nervous system diseases and they are not very effective (Jang et al., 2009; Munakata et al., 2011). Combined with special natures and multiple manners of ingestion, H₂ is a strong prospect for clinical application (Ichihara et al., 2015). In our review, we discuss the advantages and disadvantages of H₂ therapy, its underlying mechanisms, and ingestion characteristics. Furthermore, we focus heavily on H₂ therapy's relative role in treating brain diseases, since cerebral cells and tissues are sensitive to oxidative stress and

other relevant stimulation (Allen and Bayraktutan, 2009).

INGESTION CHARACTERISTICS

Under normal temperature and pressure, the solubility of H₂ is low and it cannot be largely absorbed by the body. Although humans and most mammals do not have endogenous cells that produce H₂, a large number of anaerobic bacteria in the large intestine can produce H₂ by decomposing plant fibers and carbohydrates from polysaccharide fragments. Additionally, H₂ can be expelled by anus exhaust, intestinal flora metabolism, and the respiratory tract (Levitt, 1969; Sahakian et al., 2010).

Clinically, methods for ingestion of H₂ include oral intake



of H₂ water, intravenous drip infusion of H₂-rich saline, and inhalation of air containing 2–4% H₂ gas (Ono et al., 2011; Ishibashi et al., 2012, 2014, 2015; Sakai et al., 2014). Side effects related to the concentration of H₂ are often neglected, occasionally resulting in a lack or excess of H₂ administered to patients, and, potentially, toxic effects (Nakao et al., 2010a). Research reveals that concentrations of H₂ in tissue corresponds to its concentration in the administered water or gas, suggesting that it is important to consider the disease of interest when selecting the most efficient route of H₂ administration.

MECHANISMS

Antioxygenation

Free radicals are generated during metabolic processes. By breathing 2% H₂, the free radical can be effectively removed, decreasing cerebral ischemia/reperfusion injury (Ohsawa et al., 2007). It was demonstrated at the cellular level that H₂ can selectively neutralize •OH and ONOO⁻, and therefore concluded that this selective antioxidant effect is the basis of H₂ therapy for cerebral ischemia/reperfusion injury. Additionally, a variety of animal experiments confirmed that H₂ has the capacity to improve the activity of antioxidant system, reducing damage to cells and tissues induced by oxidative stress (Xie et al., 2010; Wang et al., 2015). Other experiments showed improvement in the activity of antioxidant enzymes under H₂ inducement (Kawamura et al., 2010).

Anti-inflammation effect

Inflammation is a common pathological process accompanying most diseases, whereby activation of immunocytes and the release inflammatory cytokines are involved. This includes interleukin 1 beta (IL-1β), IL-6 and tumor necrosis factor-alpha (TNF-α). Animal experiments confirmed that ingestion of H₂ can decrease both the amount of inflammatory cytokines and immunocyte stimulation (Kawamura et al., 2010; (Kajiya et al., 2009; Liu et al., 2010; Wang et al., 2011b; Zhang et al., 2011). As a result, the degree of inflammation was alleviated through H₂ therapy.

Anti-apoptosis effect

According to recent studies, apoptosis can be triggered by intrinsic stimulation through the mitochondrial signaling pathway, or by extrinsic stimulation through cell surface death receptors (DR), such as TNFα, TNF-related apoptosis-inducing ligand (TRAIL) receptors, and Fas (CD95/APO1) (Adams, 2003; Green, 2005). In either case, activation of cysteine aspartyl proteases (caspases) is necessary, and therefore, we define apoptosis as a caspase-

dependent manner of cell death. Research showed that the apoptosis of neurons in newborn rats induced by hypoxia and ischemia is inhibited if inhaling H₂, as the ratio of Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling (TUNEL) staining positive cells and the activity of caspase-3 and caspase-12 of the hippocampus and cortex is decreased (Cai et al., 2008). Other animal studies discovered the anti-apoptosis effect of H₂. For instance, in the case of spinal cord injury, apoptosis-related indicators decline if an intraperitoneal injection of H₂-saturated saline is administered within a certain time period (Chen et al., 2010a).

Additional mechanisms

Nuclear factor-kappaB (NF-κB) is quick and widespread transcription factors in the cytoplasm that regulate expression of target genes, such as cytokines, chemokines, adhesion molecules, and oxidative stress-related enzymes. Studies based on specific animal models have demonstrated the inhibition of NF-κB with the introduction of H₂ (Wang et al., 2011a). H₂ can also control the activity of extracellular signal-regulated kinase, such as with the inhibition of phosphorylation of extracellular signal-regulated kinase1/2 (ERK 1/2) (Liu et al., 2010).

ADVANTAGES AND DISADVANTAGES

Easy preparation, low cost, non-toxicity, powerful permeability, absence of residue, are characteristics that make H₂ a strong prospect for clinical application (Ichihara et al., 2015). H₂'s powerful permeability grants it accessibility to secondary organelles, such as mitochondria and nuclei, which are primary locations of deoxyribonucleic acid (DNA) and reactive oxygen species (ROS) damage (Ohsawa et al., 2007; Nakata et al., 2015). Additionally, H₂ can selectively remove hydroxyl free radicals and nitrous acid anions. Hypoergia between H₂ and other gases when in therapeutic concentration makes H₂ gas capable of combining with other gas therapies, such as anesthesia inhalation (Nakao et al., 2010b). Furthermore, there are many methods for ingestion of H₂, such as oral intake of H₂ water, intravenous drip of H₂-rich saline, and inhalation of air containing 2–4% H₂ gas (Zheng et al., 2009; Qian et al., 2010; Lin et al., 2011; Kurokawa et al., 2015). Having various means of ingestion produces multiplicity when faced with different diseases. Unfortunately, until 2011, clinical outcomes were poor, and the effects of H₂ on human subjects are often less remarkable than those seen in animal models (Ichihara et al., 2015).

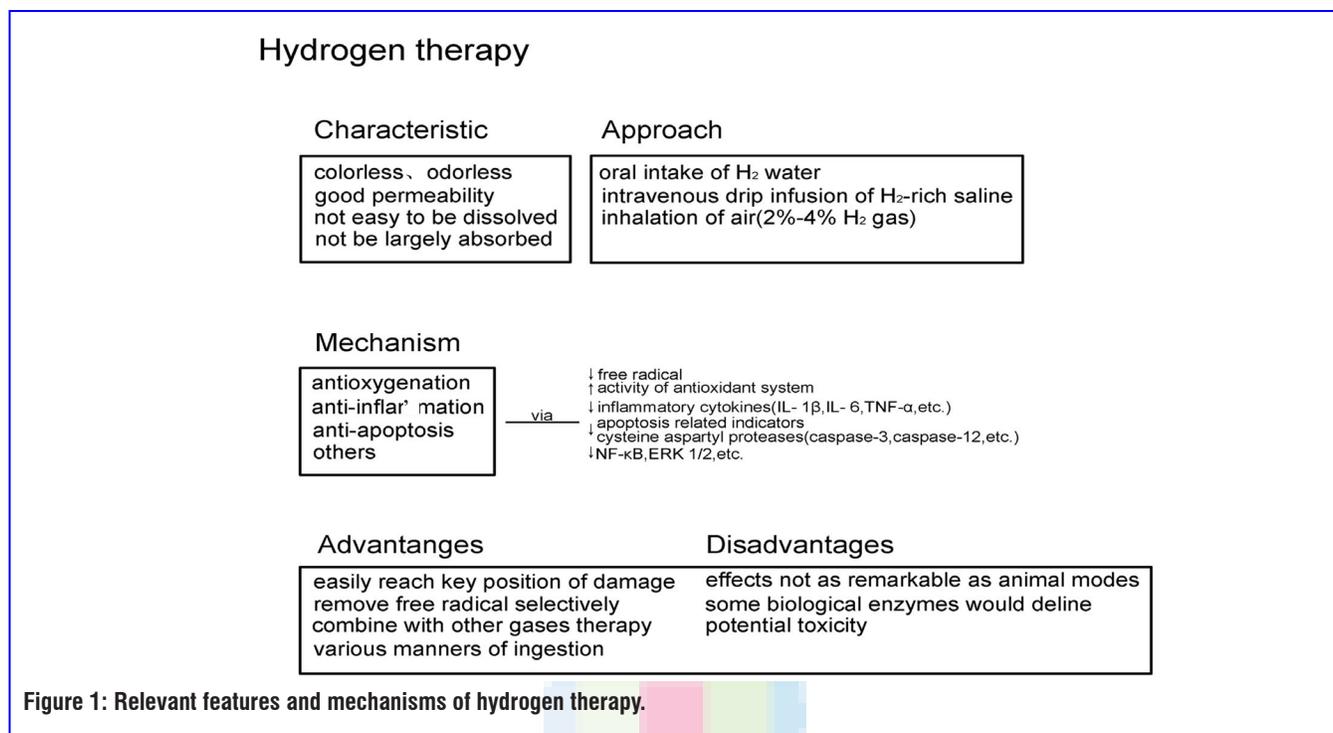
The prominent effects of H₂ play an important role in a variety of clinical diseases and animal models, but unclear and unsolved conundrums remain (Katz et al.,



2015). Research reported that levels of some biological enzymes, such as aspartate aminotransferase, alanine aminotransferase, γ -glutathione transferase and total bilirubin, declined upon ingestion of a certain amount of H_2 (Nakao et al., 2010a; Saitoh et al., 2010). These variations

and interactions were also observed in clinical trials and do not exceed standard ranges.

Intuitive understanding of H_2 therapy was reached through the summary and visual representation of the above content (**Figure 1**).



Role in cerebral diseases

Stroke

Stroke is a devastating illness second only to cardiac ischemia as a cause of death worldwide. Its main causes include cerebral vasospasm, obstacles in cerebral blood circulation, and the rupture of cerebral vessels. Oxidative stress and immunity are key elements of stroke pathobiology and are present from the stroke's early damaging stages, to the late post-ischemic tissue repair (Iadecola and Anrather, 2011; Behrouz, 2016; Sukumari-Ramesh et al., 2016). Strokes are divided into two categories: ischemic stroke and hemorrhagic stroke.

An ischemic stroke refers to local brain tissue damage caused by external or intracranial artery stenosis, or occlusion lesions sustained as a result of insufficient collateral circulation within the brain (Lyden and Zivin, 1993; Li et al., 2015). Studies show that cerebral ischemia and ischemia-reperfusion injuries contain more impact factors and mechanisms, and oxidative stress plays an important role (Allen and Bayraktutan, 2009; Hafez et al., 2014; Seifert and Pennypacker, 2014; Weaver and Liu, 2015).

One study on rats revealed that H_2 provides nerve protection in the transient middle cerebral artery occlusion (tMCAO) (Wardlaw et al., 2012). The volume of infarc-

tus, malondialdehyde (a product of lipid oxidation) and 8-hydroxy-2-deoxyguanosine (8-OHdG, a product of DNA oxidation) declined after ingesting 2% H_2 . In the same experiment, researchers also confirmed that H_2 can act as an antioxidant and selectively remove $\bullet OH$ and $ONOO^-$. Furthermore, its curative effects on cerebral ischemia/reperfusion injury were more significant than those of edaravone. H_2 also demonstrated an anti-inflammatory and anti-apoptotic effect. In addition, $TNF-\alpha$ and caspase-3 is inhibited after intraperitoneal injection of H_2 saline.

In clinical studies, upon ischemic stroke onset, 8.5–30% of patients suffer a hemorrhagic stroke (Wardlaw et al., 2012). Among patients in both the high sugar and tMCAO groups, due to its suppressive effects, this risk of brain hemorrhaging was decreased upon H_2 administration. After persistent inhalation of 2.9% H_2 for 2 hours, oxydic products and matrix metalloproteinases-9 (MMP-9) decreased, illustrating protection of the BBB (Chen et al., 2010b). Researchers speculated this effect contributed to the lower occurrence of hemorrhage accompanying cerebral infarction. Following intraperitoneal injection of H_2 saline, persistent middle cerebral artery occlusion (pMCAO) conformed to continuous ischemia of vessels, activity of antioxidant enzymes increased, and infarction areas were effectively



reduced (Nagatani et al., 2012). In animals, small doses of H₂ can significantly reduce mortality in cases of ischemic strokes that target the entire brain. Another clinical trial on brain stem infarction revealed that cooperation of H₂ and edaravone can cut down recovery time significantly better than using edaravone alone.

A hemorrhagic stroke is defined as a cerebral hemorrhage following compression and necrosis of brain tissue (Chen et al., 2015). It is also known as a hemorrhagic cerebrovascular accident, which is generally divided into two categories: subarachnoid hemorrhage (SAH) and intracerebral hemorrhage (ICH). Hemorrhagic strokes are typically more dangerous than ischemic strokes (Engelhardt and Sorokin, 2009). Microglia and inflammatory cells are activated upon hemorrhage, producing free radicals. A series of changes, like the formation of hematoma, decomposition of hemoglobin (Hb), and Fenton reactions can aggravate oxidative stress. In an ICH model for mice, inhalation of 2% H₂ for 1 hour reduced the degree of cerebral edema and improved neural function significantly, though only for 72 hours, suggesting that H₂ demonstrates only acute protection from ICH. This was speculated to be due to neutrophil infiltration and microglial activation not peaking until after 72 hours, and antioxygenation of H₂ not persistent or sufficient at that time (Manaenko et al., 2011). Lastly, infiltration and activation of mastocytes play an important role in inflammatory responses during the initial stages of stroke. H₂ was shown to protect BBB and decrease cerebral edema by preventing activation of mastocytes.

Traumatic brain injury

Traumatic brain injury (TBI) is one of the leading causes of death and disability of young people, and recovering patients usually experience impairment in learning and memory (Chua et al., 2007). Experimentation on rats showed that TBI led to brain edema, dysfunction of the nervous system, and delays in remodeling (Zhang et al., 2014). Furthermore, oxidative stress was determined an important factor in pathological changes (Zhang et al., 2014). When TBI occurred in rats, in-drawing of 2% H₂ decreased oxidation products, increased antioxidant enzyme activity, improved cerebral edema, and decreased nervous system dysfunction (Ji et al., 2010). Other studies revealed the protective effects of H₂-rich saline on hydraulic coup injury: malondialdehyde (an important antioxidant adjustment factor) declined, silent information regulators and brain-derived neurotrophic factors elevated (factors which mediate the synaptic plasticity associated with learning and memory). Additionally, Morris water maze tests also confirmed improved cognitive function following H₂ therapy (Hou et al., 2012).

Degenerative diseases

A neurodegenerative disease is characterized as the loss of cells in the brain and spinal cord, which are generally not renewable. As time progresses, neural deterioration is aggravated, leading to devastating and irreversible neural dysfunction. Neurodegenerative diseases are divided into two types: One impacting movement, such as cerebellar ataxia, and the other affecting memory and is related to dementia.

Alzheimer's disease (AD) is the most common neurological degenerative disease, in which glial cells and inflammation are activated and free radicals are produced, damaging neurons (Cupino and Zabel, 2014). One study found that drinking H₂ saline deterred the decline of age-related memory and learning ability (Gu et al., 2010). In that study, activation of cerebral 5-hydroxytryptamine and haemal antioxidant increased, resulting in a reduction of hippocampal neuron degeneration and improved scores on the Morris water maze test (Gu et al., 2010). In other AD models, NF-κB was combined with H₂ therapy (Guo et al., 2015).

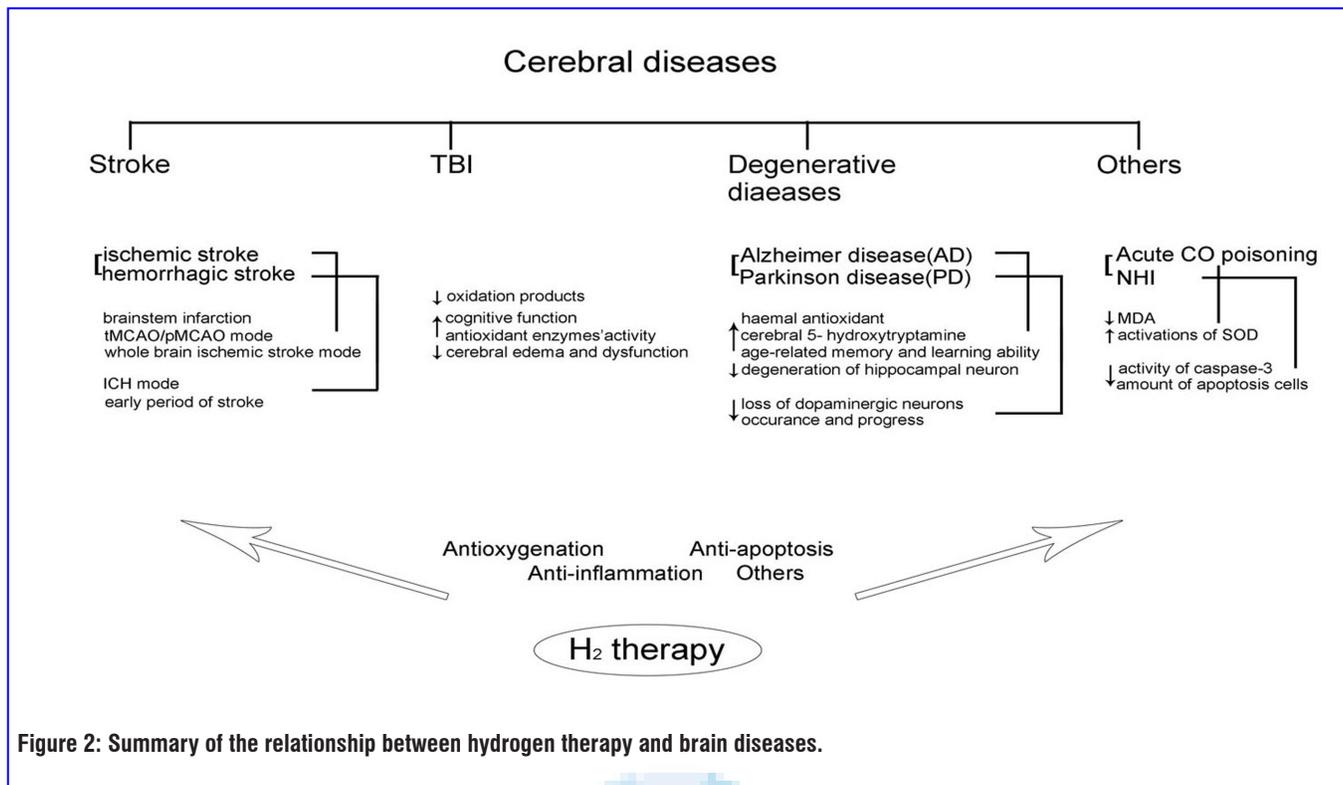
Parkinson's disease (PD) is an age-related neurodegenerative disease characterized by neural degeneration in the substantia nigra and striatum. Administration of H₂-rich saline for 6-hydroxydopamine-induced Parkinson's syndrome resulted in neural protection effects, and drinking H₂ saline yielded similar effects in 1-methyl-4-phenolic group-1,2,3,6-4 hydrogen purine-induced PD (Gu et al., 2010).

Additional cerebral diseases

Acute carbon monoxide poisoning is a systemic disease mainly involving damage of the central nervous system and delayed encephalopathy. Investigations concluded that acute brain injury and delayed encephalopathy of carbon monoxide poisoning have a close relationship with oxidative stress, cell apoptosis and immune injuries (Kao and Nanagas, 2005). Related research showed that H₂-rich saline improved activation of superoxide dismutase (SOD) in brain tissues and serum, and decreased malondialdehyde (MDA), therefore improving memory, learning, and environmental adaptation during acute carbon monoxide poisoning (Sun et al., 2011; Shen et al., 2013).

In recent years, mortality rate in premature births have increased, but effective treatments for neonatal hypoxia-ischemia (NHI) remain few. Antioxidant systems in newborns are immature and are more sensitive to free radical damage. H₂ therapy was shown useful in NHI treatment, as activity of caspase-3 and degree of cell apoptosis decreased, suggesting that H₂ offers neural protection by inhibiting apoptosis (Cai et al., 2008).

In order to facilitate simple comprehension, the relationships between H₂ therapy and the brain diseases discussed above is summarized through illustration (**Figure 2**).



DISCUSSION

Due to the feasibility and effectiveness of H₂ therapy, H₂ is a strong prospect for clinical application. Until now, studies and clinical trials of H₂ were carried out internationally and involved the investigation of a variety of diseases. In this review, we simplify comprehension of H₂ therapy and provide the basis for further clinical strategy. It is important to note that underlying mechanisms, optimal concentration, and biological safety of H₂ are worthy of deeper investigation. Additionally, the interrelationships between effects such as antioxygenation, anti-inflammation, and anti-apoptosis, remain unclear. In conclusion, we recommend more research in both the individual and molecular levels in order to drive H₂ to become a more effective therapy in clinical settings.

Abbreviations

H₂: hydrogen; •OH: hydroxyl freebase; ONOO⁻: peroxynitrite anion; BBB: blood brain barrier; IL-1β: interleukin 1 beta; TNF-α: tumor necrosis factor-alpha; DR: death receptors; TRAIL: TNF related apoptosis inducing ligand; TUNEL: Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling; NF-κB: Nuclear factor-κB; ERK 1/2: extracellular-regulated kinase1/2; DNA: deoxyribonucleic acid; ROS: reactive oxygen species; tMCAO: transient middle cerebral artery occlusion; pMCAO: persistent

middle cerebral artery occlusion; 8-OhdG: 8-hydroxy-2-deoxyguanosine; MMP-9: matrix metalloproteinases-9; SAH: subarachnoid hemorrhage; ICH: intracerebral hemorrhage; Hb: hemoglobin; TBI: Traumatic brain injury; AD: Alzheimer's disease; PD: Parkinson's disease; SOD: superoxide dismutase; MDA: malondialdehyde; NHI: neonatal hypoxia-ischemia.

Author contributions

CLL and KZ were responsible for writing the manuscript. GC was responsible for drafting and revision of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

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