Molecular hydrogen in the treatment of acute and chronic neurological conditions: mechanisms of protection and routes of administration

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Oxidative stress caused by reactive oxygen species is considered a major mediator of tissue and cell injuries in various neuronal conditions, including neurological emergencies and neurodegenerative diseases. Molecular hydrogen is well characterized as a scavenger of hydroxyl radicals and peroxynitrite. Recently, the neuroprotective effects of treatment with molecular hydrogen have been reported in both basic and clinical settings. Here, we review the effects of hydrogen therapy in acute neuronal conditions and neurodegenerative diseases. Hydrogen therapy administered in drinking water may be useful for the prevention of neurodegenerative diseases and for reducing the symptoms of acute neuronal conditions.

Oxidative stress caused by reactive oxygen species (ROS) is a major mediator of tissue and cellular injuries in various neuronal conditions, including neurological emergencies and neurodegenerative diseases.[1–7] Control of oxidative stress is a major therapeutic strategy for various neuronal conditions.[6,8,9] There are many methods for controlling oxidative stress with the use of free radical scavengers being the most common approach.[6,8] Evidence from animal experiments support the notion that free radical scavengers and antioxidants dramatically reduce cerebral damage.[9] Edaravone (MCI-186), a novel free radical scavenger, was developed to prevent lipid peroxidation in pathological neurological conditions.[8,9] Edaravone is currently the only antioxidant drug approved for treating cerebral infarction that improves the functional outcome of ischemic stroke.[8] Brain hypothermia therapy (targeted temperature management) can also effectively control oxidative stress. Brain hypothermia therapy is effective in patients with various acute neuronal diseases.[6,10,11]

In 2007, Ohsawa *et al.*[12] reported that molecular hydrogen (H_2) can act as an antioxidant to prevent and treat middle cerebral artery occlusion–reperfusion injury in rats. This effect has been supported by additional reports. Recently, the beneficial effect of H_2 has been reported in many other organs, including the brain.[13–17] The first major therapeutic effect of H_2 was that of an antioxidant, combining with hydroxyl ions to produce water.[12] Recently, other biological mechanisms of H_2 (anti-inflammatory, anti-apoptosis, anti-cytokine, DNA expression, and energy metabolism) have been proposed (Fig. 1 and 2).[18] Therefore, the biology of H_2 is not simple. In this review, we discuss the role of H_2 in various neuronal conditions.

Neurological Diseases

Ischemic brain injury. It has been reported that H_2 prevents ischemic brain damage in animal experiments.[12,19–21] Ohsawa *et al.*[12] reported that inhalation of 2% H_2 gas strongly suppressed infarct volume after middle cerebral artery ischemia–reperfusion in rats. In an electron spin resonance (ESR) study, they showed that H_2 had hydroxyl radical scavenging activity. Hydroxynonenal (HNE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) immunoreactivity was suppressed in the damaged brain after treatment with 2% H_2. H_2 inhalation reduced ischemic damage and hemorrhagic volume after transient middle crebral artery occlusion (MCAO) ischemia.[19] Free radical generation after ischemia induces matrix metalloproteinase (MMP) expression.[19,20] MMP-9 promotes hemorrhagic infarction by disrupting cerebral vessels.[20] H_2 inhalation has been found to reduce MMP-9 expression in an MCAO rat model. H_2 also has a neuroprotective effect against global ischemia. Ji *et al.*[21] reported that H_2-rich saline injection [5 ml/kg intra-peritoneal (i.p.) administration] after global ischemia reduced neuronal cell death in hippocampal Cornet d'Ammon 1 (CA1) lesions in rats. Cerebral hypoxia–ischemia and neonatal asphyxia are major causes of brain damage in neonates. H_2 gas inhalation and H_2-rich saline injection provide early neuroprotection from neonatal neurological damage.[22] Nagatani *et al.*[23] reported that that an H_2-enriched intravenous solution is safe for patients with acute cerebral infarction, including patients treated with tissue plasminogen activator (t-PA) therapy.

Metabolic syndrome is a strong risk factor of stroke. It has been reported that H_2 therapy can improve metabolic syndrome in basic and clinical settings.[24–27] H_2 therapy may reduce stroke in patients with metabolic syndrome involving diabetes mellitus.

Hemorrhagic stroke. Hemorrhagic stroke involving intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH) is a critical neuronal condition, and the mortality rate of hemorrhagic stroke is still high.[28–30] Manaenko *et al.*[28] reported a neuroprotective effect of H_2 gas inhalation using an experimental ICH animal model. H_2 gas inhalation suppresses redox stress and blood brain barrier (BBB) disruption by reducing mast cell activation and degranulation. Brain edema and neurological deficits were also suppressed. In SAH, there are several studies demonstrating

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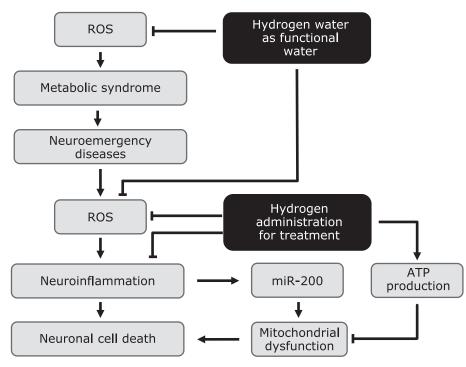J. Clin. Biochem. Nutr. | July 2017 | vol. 61 | no. 1 | 1–5

Fig. 1. Beneficial effects of molecular hydrogen in pathophysiology of various acute neuronal conditions. ATP, adenosine triphosphate; miR-200, microRNA-200; ROS, reactive oxygen species.
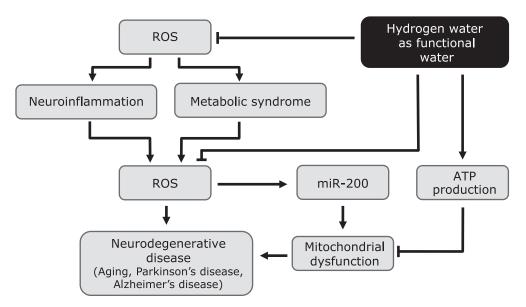

Fig. 2. Effect of consumption of hydrogen-rich water as functional water in pathophysiology of neurodegenerative diseases. ATP, adenosine triphosphate; miR-200, microRNA-200; ROS, reactive oxygen species.

Table 1. Clinical trials of molecular hydrogen in central nervous system (CNS) diseases

Disease	Hydrogen administration	Reference number
Subarachnoid hemorrhage	Intravenous infusion	(32)
Post cardiac arrest encephalopathy	2% H_2 gas inhalation	(none)
Parkinson's disease	water	(49, 50)

the neuroprotective effect of H₂ treatment.[29–31] A clinical trial has started in patients with SAH (Table 1).[32]

Traumatic brain injury (TBI). The efficacy of H₂ for treating TBI has been investigated in several studies.[18,33,34] Ji *et al.*[33] reported that in a rat TBI model, H₂ gas inhalation has been found to protect BBB permeability and regulate posttraumatic brain edema, thereby inhibiting brain damage. H₂ gas inhalation also inhibits the decrease in superoxide dismutase (SOD) activity and catalase (CAT) activity. These are antioxidant enzymes in posttraumatic brains that inhibit the production of malondialdehyde (MDA) and 8-iso-prostaglandin F2α (8-iso-PGF2α). Eckermann *et al.*[34] reported that in a surgical trauma mouse model involving right frontal lobectomy, H₂ gas inhalation has been found to inhibit postoperative brain edema and improve the postoperative neurobehavioral score. The same report also showed that lipid peroxidation and the production of oxidative stress substances were not inhibited by H₂ gas inhalation.[34] The therapeutic effect of H₂-rich water following TBI and in posttraumatic onset of Alzheimer's disease (AD) was investigated by Dohi *et al.* in 2014,[18] who investigated whether the consumption of H₂-rich water 24 h prior to trauma can inhibit neuronal damage in a controlled cortical injury model using mice. The authors found that the expression of the phosphorylated tau proteins AT8 and Alz50 in the hippocampus and cortex was blocked in mice that consumed H₂-rich water. Moreover, the activity of astrocytes and microglia were inhibited in mice TBI model consuming H₂-rich water. The expression of genes induced by TBI, particularly those that are involved in oxidation/carbohydrate metabolism, cytokine release, leukocyte or cell migration, cytokine transport, and adenosine triphosphate (ATP) and nucleotide binding, was inhibited by consuming H₂-rich water. Dohi *et al.*[18] specifically reviewed the role of H₂-rich water in neuroinflammation following brain trauma. The consumption of H₂-rich water influenced the production of cytokines and chemokines in the damaged brain and inhibited the production of hypoxia inducible factor-1 (HIF-1), MMP-9, and cyclophilin A. However, H₂-rich water did not affect the production of amyloid precursor protein (APP), Aβ-40, or Aβ-42. They also investigated the relationship between H₂ and ATP production and reported that H₂ increased basal respiration, reserve capacity, and nonmitochondrial respiration but did not increase aerobic ATP production. It has thus been demonstrated that the inhibitory effects of H₂ on nerve damage are not solely due to its simple function as a free radical scavenger (Fig. 1 and 2).

Spinal cord injury. Chen *et al.*[35] reviewed the effects of H₂-rich saline administration (i.p.) in a rat traumatic spinal cord injury model. They found that posttraumatic neurological symptoms were improved by H₂-rich saline treatment. Furthermore, H₂-rich saline treatment has been found to reduce inflammatory cell infiltration, TdT-mediated dUTP nick and labeling (TUNEL)-positive cells, and hemorrhage. In addition, oxidative stress was inhibited and the expression of brain derived neurotrophic factor (BDNF) was increased. The effects of H₂ administration on spinal cord ischemia have also been reported.[36,37] Huang *et al.*[36] investigated the effects of H₂ gas inhalation in a rabbit spinal cord ischemia–reperfusion model. They reviewed the effects of H₂ inhalation with different concentrations (1, 2, and 4%) and reported that H₂ gas inhalation at concentrations of 2% and 4% inhibited neuronal death. However, they did not observe significant differences between the two groups in terms of effects with 2% and 4% being equally effective.[36] It has been reported that the inhalation of 2% H₂ gas inhibits apoptosis following spinal cord injury caused by ischemia–reperfusion. In addition, H₂ gas inhalation regulates caspase-3 activity, the production of inflammatory cytokines, oxidative stress, and the decrease in endogenous antioxidant substances. Zhou *et al.*[37] also reported that H₂-rich saline administration (i.p.) has beneficial effects on spinal cord ischemia–reperfusion injury in rabbits.

Other acute neurological conditions. In recent years, research has shown that there is a high incidence of comorbid central nervous system symptoms in sepsis cases.[38] Using a mice cecal ligation and puncture (CLP) model, Liu *et al.*[39] reported that H₂ gas inhalation improves septic encephalopathy. They reported that 2% H₂ gas inhalation inhibited post-CLP apoptosis, brain edema, BBB permeability, cytokine production, and oxidative stress in the CA1 hippocampus region as well as improves cognitive function. Nakano *et al.*[40] reported that maternal administration of H₂ has a suppressive effect on fetal brain injury caused by intrauterine inflammation with maternal intraperitoneal injection of lipopolysaccharide (LPS).

The treatment of carbon monoxide (CO) poisoning encephalopathy, which is a common gas poisoning, is yet to be established.[41,42] Sun *et al.*[42] and Shen *et al.*[41] investigated the effects of H₂-rich saline. They reported that in a CO poisoning model, the administration of H₂-rich saline decreased glial activation, cytokine production, oxidative stress, and caspase 3 and 9 production as well as inhibited nerve cell death.

It is known that stress causes nerve cell impairments.[43] The consumption of H₂-rich water inhibits oxidative stress and thereby inhibits the onset of stress-induced brain damage.[43]

Hypoxic brain injury caused by asphyxiation, hypoxic ischemic encephalopathy, neonatal asphyxia, and other similar hypoxia-mediated event is a common clinical condition in medical emergencies. H₂ treatment has been found to inhibit cell death in an *in vitro* hypoxia/reoxygenation model using immortalized mouse hippocampal (HT-22) cells. H₂ treatment increased phosphorylated Akt (p-Akt) and B-cell leukemia/lymphoma-2 (BCL-2), while it decreased Bax and cleaved caspase-3.[44] In recent years, it has been found that the microRNA-200 (miR-200) family regulates oxidative stress.[44] The inhibition of miR-200 suppresses H/R-induced cell death, reducing ROS production and MMP. H₂ treatment suppressed H/R-induced expression of miR-200. In Japan, a double blind randomized controlled trial for post cardiac arrest syndrome has started from 2017 (Table 1).

Neurodegenerative Diseases

Parkinson's disease (PD). PD is a disorder that presents with extrapyramidal symptoms caused by the degeneration and loss of dopamine-producing cells in substantia nigra. Oxidative stress is known to be involved in the clinical condition of PD.[7] Moreover, the involvement of mitochondrial dysfunction in PD has been reported.[45] The effects of H₂ on PD have been reported in animal models of PD as well as in clinical studies.[46–48] In 2009, Fujita *et al.*[47] and Fu *et al.*[48] reported that consuming H₂-rich water inhibits oxidative stress on the nigrostriatal pathway and prevents the loss of dopamine cells in a PD animal model. With the consumption of H₂-rich-water-drinking, oxidative stress in the nigrostriatal pathway was inhibited and loss of dopamine cells was decreased. These results suggest that consuming H₂-rich water could affect the onset of PD. In recent years, the results of a clinical trial on the effects of consuming H₂-rich water for PD have been reported.[49] A randomized double-blind study showed that consuming H₂-rich water (1,000 ml/day) for 48 weeks significantly improved the total Unified Parkinson's Disease Rating Scale (UPDRS) score of PD patients treated with levodopa. A double-blind multi-center trial of H₂ water is currently underway (Table 1).[50]

Alzheimer's disease (AD). AD, an age-related neurodegenerative disease, is the most common cause of dementia.[1,51] Pathologically, it is characterized by the deposition of Aβ protein outside nerve cells and the accumulation of phosphorylated tau protein inside nerve cells. There is also a marked loss of nervous cells in the cerebral cortex.[52] In recent years, oxidative stress and neuroinflammation have been reported to be involved in AD.[1,5] To date, reports have centered on the involvement of oxidative

stress in brain parenchyma.[1,51,53] The accumulation of Aβ protein is strongly associated with the failure of Aβ clearance that is closely related to the pathogenesis of AD.[5] It is known that low-density lipoprotein receptor-related protein 1 (LRP1) is involved in Aβ protein elimination. LRP dysfunction caused by oxidative stress and neuroinflammation is involved in the onset of AD.[5] The regulation of oxidative stress and neuroinflammation may prevent the onset or progression of AD. A number of reports have investigated the effects of H_2 for the prevention of AD onset.[51,53] In a rat AD model, it has been reported that the administration of H_2-rich saline (5 ml/kg, i.p., daily) inhibited oxidative stress, cytokine production, and nuclear factor-κB (NF-κB) production in the hippocampus and cerebral cortex, and improved impaired memory.[51,53] It has also been reported that consuming H_2-rich water inhibits age-related brain alterations and spatial memory decline.[54]

Method and Route of Administration in H_2 Therapy

As a small (2 Da), uncharged molecule H_2, would be expected to readily distribute throughout the body, including being able to easily penetrate cell membranes, However we are unable to determine the distribution of H_2 among organs and its concentrations in each organ and serum based on the administration methods and dosage. This problem was investigated in 2014.[55] A comparative review was conducted on the consumption of H_2-rich water, i.p. or intravenous administration of H_2-rich saline, and inhalation of H_2 gas. The results showed that the highest concentrations are reached 1 min after intravenous administration and 5 min after oral administration. The highest concentration was reached 30 min after the inhalation of H_2 gas and was maintained for some time. Although H_2 concentrations in the brain tend to be high after either intravenous administration or inhalation, no significant differences have been observed in comparison with the concentrations after the consumption of H_2-rich water and i.p. administration of H_2-rich saline. Thus, although there have been variations based on the administration method, all methods have been found to result in the presence of H_2 in the serum and brain tissue. Liu et al.[39] measured H_2 levels in the arteries, veins, and brain tissues after the inhalation of 2% H_2 gas. They found that arterial H_2 peaked at 30 min after administration, whereas venous and brain tissue H_2 peaked at 45 min after administration. They reported that H_2 levels were similar in arteries and brain tissues. This demonstrated that H_2 migrates to the brain tissue regardless of the method of administration. These results suggest that the consumption of H_2-rich water prevents neurodegenerative disease and that H_2-rich drinking water could be used to treat acute brain disorders (Fig. 1 and 2).

Conclusions

We have examined the effects of H_2 treatment on acute central nervous system diseases and on chronic neurodegenerative diseases. We have also examined the various mechanism by which H_2 exerts its neuroprotective effects H_2 acts as a scavenger for OH^- and $ONOO^-$, affects neuroinflammation, preserves mitochondrial energy production, and possesses neuroprotective properties. Unlike more conventional drugs, H_2 treatment, particularly the consumption of H_2-rich water, has no known serious side effects and is effective for preventing the onset of neurodegenerative disease and aggravation of acute neuronal conditions.

Acknowledgments

Abbreviations

AD	Alzheimer's disease
APP	amyloid precursor protein
ATP	adenosine triphosphate
BBB	blood brain barrier
CA1	Cornet d'Armon 1
CLP	cecal ligation and puncture
CO	carbon monoxide
ICH	intracerebral hemorrhage
LRP	lipoprotein receptor-related protein
MCAO	middle cerebral artery occlusion
miR-200	microRNA-200
MMP	matrix metalloproteinase
PD	Parkinson's disease
ROS	reactive oxygen species
SAH	subarachnoid hemorrhage
TBI	traumatic brain injury

Conflict of Interest

No potential conflicts of interest were disclosed.

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