

Multiple Effects of Molecular Hydrogen and its Distinct Mechanism

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Abstract

Molecular hydrogen (H₂) has been reported to be effective for a variety of disorders and its effect has been ascribed to a selective scavenge of hydroxyl radicals (•OH) at the beginning. Consumption of H₂ was either by inhalation, drinking H₂-containing water (H₂ water), or infusion of H₂-containing saline. Among various disorders, animal model of ischemic injury and Parkinson's disease showed significant amelioration after H₂ treatment. The mechanism of neuroprotection, however, is not simple. Multiple mechanisms may exist to produce acute and chronic effect. For chronic effect, H₂-induced neuroprotection takes several days to develop and lasted several days, suggesting that H₂ may work as a modulator of signal transduction as indicated by allergy model. The evidence that drinking H₂ water was the most effective way rather than inhaling H₂ in Parkinson's disease model animal led to the finding that H₂ induces ghrelin production and release from the stomach by activating β1 adrenergic receptors. The distinct mechanism due to the brain-stomach connection may help to understand the broad spectrum of H₂ function. In addition, clinical trials have shown promising results.

Keywords: Molecular hydrogen; Neuroprotection; Parkinson's disease; Brain-stomach connection; Ghrelin

Abbreviations:

ASK1: Apoptosis Signal-Regulating Kinase 1; BBB: Blood-Brain Barrier; COX2: Cyclooxygenase 2; H₂: Molecular Hydrogen; NO: Nitric Oxide; IFNγ: Interferon γ; iNOS: Nitric Oxide Synthase; i.p: Intraperitoneally; I/R: Ischemia-Reperfusion; DSS: Dextran Sulfatesodium; LPS: Lipopolysaccharide; MPP+: 1-Methyl-4-Phenylpyridinium Ion; MPTP: 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine; NOX: NADPH Oxidase; •OH: Hydroxyl Radicals; 6-OHDA: 6-Hydroxydopamine; PCA: Passive Cutaneous Anaphylaxis; PD: Parkinson's Disease; QOL: Quality Of Life; ROS: Reactive Oxygen Species; SNpc: SN parscompacta; s.c: Subcutaneously; TBI: Traumatic Brain Injury; TH: Tyrosine Hydroxylase; TLR4: Toll Like Receptor 4; TNF-α: Tumor Necrosis Factor-α

Introduction

Molecular hydrogen (H₂) was first documented by Philippus Aureolus Paracelsus in 1520 as a flammable gas, though it did not have an official name yet. As described by Dixon et al. [1], the history of H₂ showed that the first use of H₂ was as a balloon carrier. Then it was used in an aircraft and now H₂ becomes one of the most promising energy sources for future vehicle without pollution [2]. Not only in energy field, H₂ is now getting more and more attention as a useful and unique medical gas; H₂ has been reported to be effective for a variety of disorders. Its effect has been ascribed to a selective scavenger of hydroxyl radicals (•OH) which is highly toxic dangerous radical among reactive oxygen species (ROS). Antioxidant-acute ischemic model was first reported in 2007 [3]. Since then, rapidly growing fields

by increased number of papers on protective role of H₂ are shown and effective disorders are well documented [1,4-6]. Also, H₂ as a novel antioxidant to efficiently reduce oxidative stress was reported with potential for the improvement of mitochondrial diseases [7] and ischemia-reperfusion injury [8]. H₂, therefore, is a small molecule that is easily produced. It seems to be a beneficial gas with no side effects reported thus far.

Though H₂ was recently reported as an effective antioxidant, it is becoming clear that there is not only ROS-scavenging as an acute effect but also a chronic effect accompanying transcriptional alterations and gene expression [9,10]. For example, in neurodegenerative diseases such as Parkinson's disease [11], ingestion of H₂ in drinking water was the most effective rather than inhaling H₂ [12]. Importantly the concentration of H₂ in drinking water was substantially low [13]. Consequently, increase in the concentration of H₂ in the brain was not detected [13]. Recent finding that H₂ in drinking water induces ghrelin release from stomach [14] suggest a molecular mechanism underlying marked effects of small amount of H₂. In addition, in case of traumatic brain injury (TBI), not only acute changes such as edema but also cytokine release in chronic phase were attenuated by drinking H₂ containing water. It was shown that gene expression related to oxidation/carbohydrate metabolism after TBI was reversed by drinking H₂ containing water [15].

Since clinical studies have recently started and are showing promising results, H₂ as a molecular gas will become a good subject in translational research for various pathologies.

Does Hydrogen Work as an Antioxidant?

In 2007, it was reported that H₂ dose-dependently reduces •OH *in vitro*, whereas H₂ was too weak to reduce physiologically important

ROS such as NO• and superoxide [3]. Therefore, H₂ seems to act as a specific antioxidant against toxic •OH. It was observed that H₂ in medium protected culture cells against •OH, which was produced by treatments of cells with antimycin A (mitochondrial respiratory complex III inhibitor), fenton reaction reagents and X-irradiation, respectively [3,16]. H₂ is the smallest molecule in the universe. Therefore, it has a unique ability to rapidly diffuse across membrane; it can reach and react with cytotoxic •OH in all organelles including mitochondria and nucleus, and thus effectively protect cells against oxidative damage.

To examine the therapeutic applicability of H₂ at first, a rat model of ischemia was used [3]. Rats inhaled 2% of H₂ gas during focal ischemia by occluding middle cerebral artery for 90 min and reperfusion for 30 min. One day after ischemia-reperfusion (I/R), a clear H₂-dependent decrease in infarct volume of the brain was observed. The neuroprotective effect of H₂-loaded eye drops on retinal I/R injury [17] was also explored. To produce ischemia, intraocular pressure was elevated and H₂-loaded eye drops during 90 min of I/R process. After 7 days, I/R induced a marked thinning and atrophy of the retina; however, H₂-loaded eye drop-treated retina showed a nearly normal structure without glial activation. After reperfusion, acute and greater production of •OH injures each organ. It was considered that H₂ detoxifies •OH and reduces the damage of I/R. Protective effects of H₂ against I/R injury have been already reported in the brain, eye, heart, liver, kidney and intestine.

The possibility of H₂ molecule as a radioprotector was also examined [16]. Within picoseconds, radiation lyses water and produces •OH. Within seconds, radiation directly damages DNA and •OH oxidizes biomolecules. These unfavorable changes lead to cell death and genomic instability that causes cancer and birth defects and affects future generation. The lung is known as one of the most susceptible organs to radiation injury. During x-irradiation on the lung, mice were treated with 3% H₂ gas. After that, they ingested H₂ water ad libitum. One week after irradiation, apoptotic cell death was reduced in irradiated mice treated with H₂. Five months after irradiation, the late phase of damage was checked: Chest micro-CT showed that irradiated mice had a regional increase of radio opaque area indicating lung lesions, whereas H₂-treatment ameliorated them. These results indicate that H₂ works as an antioxidant in both short- and long-term way.

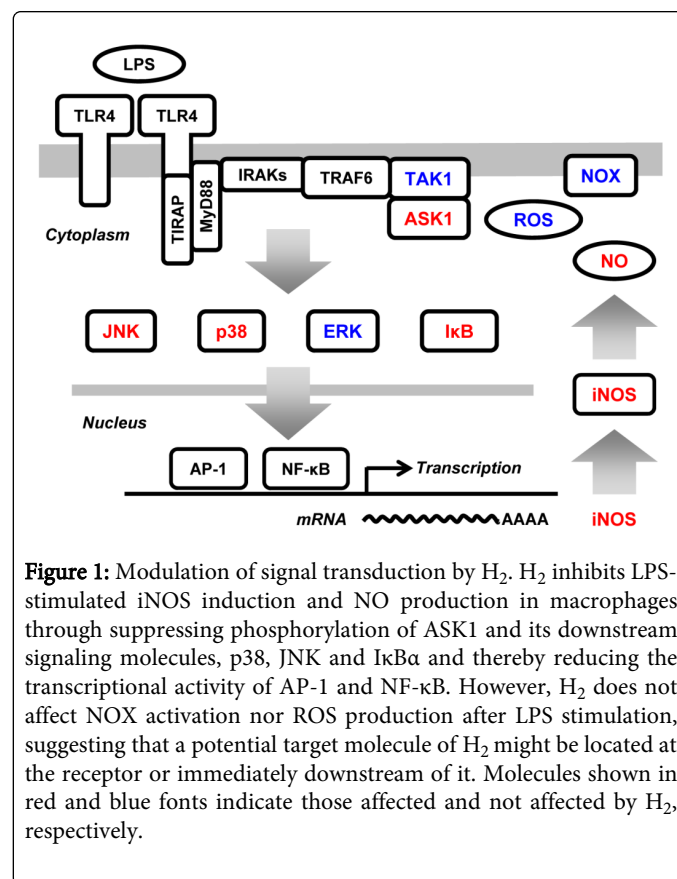
Hydrogen Works as a Signal Modulator

Reduction of oxidative stress has been confirmed in various oxidative stress-related disease models. However, some effects of H₂ are not causally associated with the effects as a direct antioxidant as mentioned above. Another example is that H₂ inhibits Type I allergy or immediate-type hypersensitivity, which proposed “modulation of signal transduction” as another mechanism of the H₂ effects [18].

In a mouse model of Type I allergy, drinking H₂ water attenuated passive cutaneous anaphylaxis (PCA) reaction and reduced the increase in serum histamine levels after the antigen challenge. In IgE-sensitized basophilic leukemia RBL-2H3 cells pretreatment with H₂ reduced the antigen-induced release of β-hexosaminidase, a marker of degranulation. H₂ inhibited antigen-induced phosphorylation of Lyn and its downstream signaling molecules including Syk, PLCγ1, PLCγ2, Akt, ERK1/2, JNK, p38 and cPLA2, as well as antigen-induced intracellular calcium increase. H₂ also inhibited antigen-induced activation of NADPH oxidase (NOX) and production of ROS, which

was considered to be most likely a consequence of the suppression of signal transduction. These results suggested that H₂ water could ameliorate type I allergy such as bronchial asthma, rhinitis, conjunctivitis and urticaria and that H₂ inhibits allergic reactions not by its radical scavenging activity but by modulating specific signaling pathways.

Previous reports have demonstrated that H₂ attenuates inflammation in animal models of inflammatory diseases such as zymosan-induced inflammation [19] and inflammatory bowel disease [20], but the underlying molecular mechanisms have not yet been elucidated. As shown in Figure 1, lipopolysaccharide (LPS) binding to its receptor, toll like receptor 4 (TLR4), initiates signal transduction, resulting in the activation of transcription factors such as AP-1 and NF-κB and the transcription of inflammatory genes such as inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2). Increased expression of iNOS leads to increased production of NO (nitric oxide) and an excess amount of NO plays a deleterious role in inflammation. We hypothesized that H₂ might inhibit inflammatory responses through modulating the signal transduction and examined the hypothesis in LPS/interferon (IFN) γ-stimulated macrophage RAW264 cells [21].



The results showed that treatment with H₂ suppressed LPS/IFNγ-stimulated iNOS induction and NO production in macrophages. H₂ also inhibited LPS/IFNγ-stimulated phosphorylation of apoptosis signal-regulating kinase 1 (ASK1) and its downstream signaling molecules, p38 and JNK, as well as IκBα. However, H₂ did not affect NOX activation nor ROS production after stimulation. Based on these results, it was speculated that a potential target molecule of H₂ might be located at the receptor or immediately downstream of it. It was also

demonstrated that oral intake of H₂-rich water alleviated anti-type II collagen antibody-induced arthritis in mice, a model for human rheumatoid arthritis, the finding of which has been supported by a clinical study [22].

The inhibitory effects of H₂ on LPS/IFN γ -stimulated NO release from immortalized murine microglia (BV-2 cell line) and primary cultured microglial cells (unpublished data) [23], suggest that H₂ may protect against neurodegenerative diseases such as AD and PD in part through inhibiting neuroinflammation. It was also reported that the protective effects of drinking H₂ water in LPS-induced sickness behavior are associated with a shift towards anti-inflammatory gene expression profile at baseline (down-regulation of TNF- α and upregulation of IL-10). In addition, H₂ increases the amplitude, but shortens the duration and promotes the extinction of neuroinflammation. Consistently, H₂ modulates the activation and gene expression in a similar fashion in BV-2 cells, suggesting that the effects observed *in vivo* may involve the modulation of microglial activation [10].

Taken together, it was proposed that H₂ may act not only as an antioxidant but also as a signal modulator. In support of this hypothesis, it has been recently reported that H₂ inhibits signal transduction in animal models of acute liver injury [24] and amyloid- β -induced Alzheimer's disease (AD) [25].

Are Concentrations of Hydrogen in Water and in the Brain Important?

Ingestion of H₂ in drinking water protected dopaminergic neurons in PD model animals as well [13,26]. To discuss about the neuroprotective effects of H₂ water in the central nervous system, there has been a fundamental question whether or not H₂ in drinking water goes into the brain and works as antioxidant. Increase in H₂ concentration in arterial and venous blood in rats were observed after inhalation of H₂ and 30% O₂ for 1 h under the anesthetics N₂O and halothane [3]. However, apparent increase in H₂ concentration in the brain has not been observed.

The question whether or not H₂ reaches to the brain was tested using H₂ electrode (teflon-coated platinum electrode with 2 mm bare tip) which was inserted through guide-cannula into right striatum of rats. Inhaling H₂ gas with air immediately increased H₂ concentration in the striatum and disappeared within 10 min after stopping inhaling [13]. Likewise, there was no apparent increase in H₂ concentration in the striatum with neither drinking of saturated H₂ water in free-moving rats nor putting saturated H₂ water into to the stomach in anaesthetized rats. These results suggest that H₂ concentration in the brain supplied by drinking H₂ water is too low to detect, even if it goes into the brain.

Another critical point is that H₂ in drinking water did not show any concentration-dependence in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model animals. It was all or none type of response and 0.08 ppm, which was one twentieth of saturated concentration (1.6 ppm) under the pressure of normal atmosphere. Water containing 0.08 ppm H₂ showed maximum effect and water with even higher concentration of H₂ did not show any bigger effects [13]. Lack of concentration-dependence of H₂ was also reported in another model of PD, a 6-hydroxydopamine(6-OHDA)-induced PD model [12]. These results suggest that high concentration of H₂ in water (~5 ppm), which could be made with additional pressure, would be no use at least in PD model animal. Further studies

are required to prove whether the lack of dose response is disease-specific or not.

Hydrogen Production in the Intestine

As mentioned above, low concentration of H₂ in drinking water is quite effective in various disease models. On the other hand, endogenous H₂ gas is produced in our intestine by bacteria. In addition, H₂ can be produced as a byproduct when lactulose, a synthetic sugar that is made of fructose and galactose, is digested by bacteria in the colon [27]. Breath-hydrogen production after oral glucose administration was examined [28]. Hydrogen breath test after oral administration of lactulose is clinically applied to examine small intestinal bacterial overgrowth, which is an underlying mechanism of irritable bowel syndrome [29]. Since lactulose is able to ameliorate dextran sulfate sodium (DSS)-induced intestinal inflammation in rats [30] and the effect of lactulose on DSS-induced colitis can also be ascribed to H₂ production in the colon, it was hypothesized that lactulose potentially ameliorates cerebral infarction by producing intestinal hydrogen [31]. Similarly, it is speculated that lactulose may abolish development of parkinsonian symptoms in a rat model of 6-OHDA-induced PD as well. Since drinking a large amount of water is not easily accommodated by PD patients, it will be easier to increase breath H₂ levels if it works.

The result was that lactulose efficiently increased breath hydrogen levels in healthy subjects, PD patients, and rats. However, lactulose marginally ameliorated development of PD in rats. It was also demonstrated that continuous inhalation of 2% H₂ gas had marginal effects, whereas intermittent inhalation had variable but over effects on prevention of PD in rats [12]. Like above, the evidence that H₂ in drinking water was the most effective way for neuroprotection.

Acute and Chronic Effect of Hydrogen with Different Mechanism

Acute and semi-acute effects

The most typical example of acute effect of H₂ as an antioxidant would be the neuroprotective effects in I/R model by inhaling 2% H₂ [3]. Neuroprotective effects in PD models may be also explained by antioxidant. Two major mechanisms are proposed as causes of PD: one is excessive oxidative stress and the other is abnormal ubiquitin-proteasome system [32,33]. The neurotransmitter, dopamine, is a prooxidant *per se* and dopaminergic cells are continuously exposed to high concentrations of radical oxygen species derived from dopamine. Dopaminergic neurons are thus destined to cope with a radical-producing dopamine.

The first PD model used in H₂ research was a rat model of hemi-PD by stereotactically injecting catecholaminergic neurotoxin, 6-OHDA, in the right striatum [26]. *Ad libitum* administration of H₂ water starting one week before surgery completely abolished the development of hemi-Parkinson's symptoms. It was also started to give H₂ water three days after surgery, and hemi-Parkinson's symptoms were again suppressed, but not as much as those observed in pre-treated rats. Pre-treated rats were sacrificed 48 hr after toxin injection, and the tyrosine hydroxylase (TH) activity at the striatum was decreased in both H₂ and control groups, which indicated that H₂ did not directly detoxicate 6-OHDA, but exerted a delayed protective effect for dopaminergic cells.

Another toxin, MPTP, is a protoxin which is high lipophilic molecule. MPTP can penetrate the blood-brain barrier (BBB), being readily converted to 1-methyl-4-phenylpyridinium ion (MPP⁺). Dopaminergic neurons exhibit a high-affinity uptake process of MPP⁺ through the dopamine transporter, which allows the neurotoxin MPP⁺ to cause selective dopaminergic neuronal loss [34]. Inside the neurons, MPP⁺ accumulates in the mitochondrial matrix, and impairs mitochondrial respiration by inhibiting the multi-subunit enzyme Complex I of the mitochondrial electron transport chain [35,36]. Inhibition of complex I causes two early and major events: ATP depletion, and the buildup of ROS.

The extents of loss of dopaminergic neurons and behavioral alteration vary depending on differences in the protocol of MPTP administration, acute administration (20 mg/kg, 3 or 4 times at 2 hours interval) and chronic (continuous) administration model infusing subcutaneously (s.c.) or intraperitoneally (i.p) using osmotic pumps. As the protective mechanism, MPTP-induced accumulation of cellular 8-oxoguanine (8-oxoG), a marker of DNA damage, and 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation, were significantly decreased in the nigro-striatal dopaminergic pathway in mice drinking H₂ water [13]. These results may suggest that H₂ works as antioxidant.

H₂-rich saline was also reported to have protective role in the brain. H₂ inhalation had been reported to be beneficial to traumatic brain injury (TBI) via reducing oxidative stress [37] but the idea was that H₂-rich saline might be more suitable for clinical application. TBI-challenged rats exhibited significant brain injuries characterized by the increase of blood-brain barrier (BBB) permeability, brain edema, and lesion volume as well as neurological dysfunction. Those symptoms were dose-dependently ameliorated by H₂-rich saline intraperitoneally administered at 5 min after TBI [38]. Therefore, it was suggested that H₂ may be a more effective therapeutic strategy for TBI patients.

H₂-rich saline may also effectively protect the brain from injury after acute CO poisoning [39]. This study showed that administration of H₂-rich saline by peritoneal injection (6 mL/kg/24 h) improved the cognitive deficits and reduced the degree of necrosis, apoptosis, and cell autophagy in rats. The mechanism of this protection may be related to reducing oxidative stress and consequently lessening oxidative damage by affecting trace elements *in vivo*. It was also reported that H₂-rich saline ameliorates the retina against light-induced damage in rats [40].

These results, if not all, were all in line with the facts that H₂ works as an antioxidant. On the other hand, there was a discrepancy as to the mechanism how H₂ could be an antioxidant. If drinking H₂ water does not cause detectable increase in H₂ concentration in the brain as mentioned above, it may not be due to a direct effect as a ROS scavenger as was observed *in vitro* system.

Chronic effect

It was obvious that one-day drinking H₂ water was not enough to show any protective effect in MPTP-induced PD model. In addition to anti-oxidative effect, H₂ may protect against AD and PD in part through inhibiting neuroinflammation. If it's time-dependent effect, how many days of drinking H₂ water is necessary? To investigate this, different period of drinking H₂ water was tested (1, 3 and 7 days) using acute MPTP-induced PD model mice (Figure 2A). After these so-called pre-treatment with H₂ water, the brains were investigated 7 days after MPTP-injection. During the 7 days before examination of the

brains, non-H₂ water (control water) was given to the animals so that actual H₂ did not exist in the animals. The significant neuroprotection was observed after 7 days of drinking H₂ water (Figure 2B).

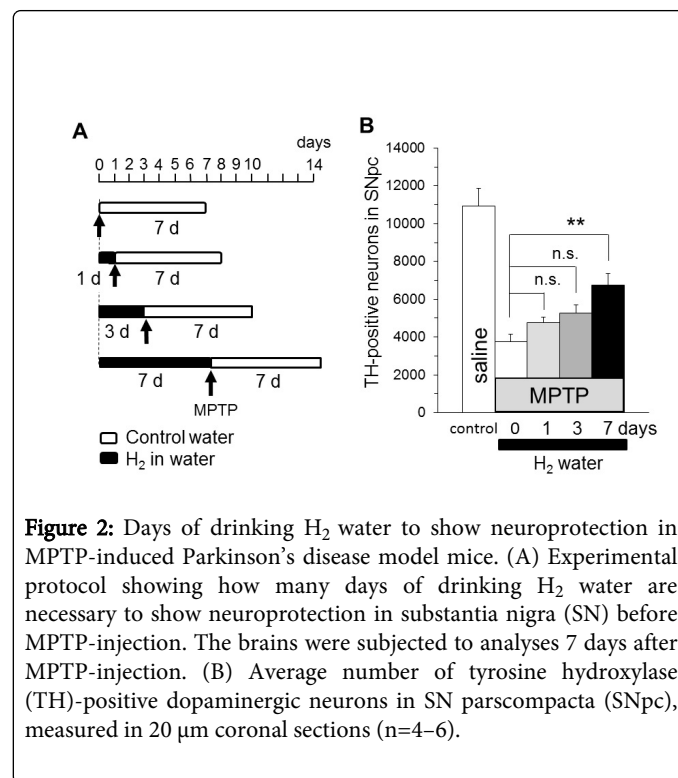


Figure 2: Days of drinking H₂ water to show neuroprotection in MPTP-induced Parkinson's disease model mice. (A) Experimental protocol showing how many days of drinking H₂ water are necessary to show neuroprotection in substantia nigra (SN) before MPTP-injection. The brains were subjected to analyses 7 days after MPTP-injection. (B) Average number of tyrosine hydroxylase (TH)-positive dopaminergic neurons in SN parscompacta (SNpc), measured in 20 μm coronal sections (n=4–6).

If the pre-treatment of H₂ water needs ~7 days to show neuroprotection, how many days does the protective effect last even after stopping H₂ water consumption? To answer this question, after 7 days of H₂ water, 1, 3, and 7 days of non-H₂ water (control water) was supplied to the animals, then MPTP was injected. As in Figure 3A, further 7 days of control water application was followed before the examination of the brain. With 3 days of non-H₂ water before MPTP injection, the neuroprotective effect of H₂ decreased nearly to one third. As a result, neuroprotective effect obtained by 7 days of drinking H₂ water could last only a couple of days (Figure 3B).

Similar phenomena were also observed in ischemic protection of optic nerves, a model of myelinated central nervous system white matter. The protective effects took several days to develop, lasted several days and provided partial protection in a novel manner from what has been previously described [41]. These observations raise intriguing therapeutic and preventing options.

The molecular mechanism or changes in gene expression under these chronic effects are still under investigation. Part of the effects may be due to a modulation of signaling as described above, consequently inhibiting neuroinflammation. Or the change in gene expression related to oxidation/carbohydrate metabolism was reversed by drinking H₂ water as was observed in TBI model [15].

Distinct Mechanism of H₂-Induced Neuroprotection via Stomach-Brain Connection

A distinct mechanism of H₂-induced neuroprotection was recently shown [14]. The fact that H₂ in drinking water was the most effective [12] gave a hint that there would be a link between digestive organs

and the brain. The hypothesis was that oral H₂ induces a messenger molecule, which travels to the brain and exerts neuroprotective activity. The results were that oral hydrogen water induces ghrelin gene expression in the stomach and increased plasma ghrelin levels, taking 4 days [14]. Ghrelin is a growth hormone (GH)-releasing peptide and an endogenous ligand specific for growth-hormone secretagogue receptor (GHS-R) [42]. GHS-R is highly expressed by dopaminergic neurons of the substantia nigra [43], and has been suggested that ghrelin protects nigrostriatal dopamine neurons via an uncoupling protein 2 (UCP2)-dependent mitochondrial mechanism [44,45].

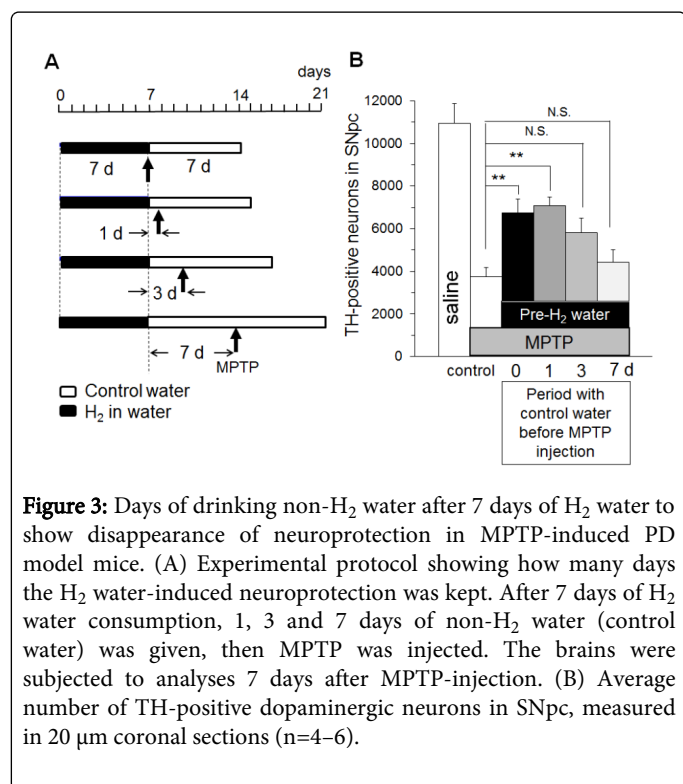


Figure 3: Days of drinking non-H₂ water after 7 days of H₂ water to show disappearance of neuroprotection in MPTP-induced PD model mice. (A) Experimental protocol showing how many days the H₂ water-induced neuroprotection was kept. After 7 days of H₂ water consumption, 1, 3 and 7 days of non-H₂ water (control water) was given, then MPTP was injected. The brains were subjected to analyses 7 days after MPTP-injection. (B) Average number of TH-positive dopaminergic neurons in SNpc, measured in 20 μm coronal sections (n=4-6).

As a mechanism of increasing ghrelin gene expression, it has been reported that β₁-adrenergic receptor stimulation increases ghrelin secretion *in vitro* and *in vivo* [46,47]. The neuroprotective effects of H₂ water was cancelled either by specific β₁-blocker, atenolol, or ghrelin receptor antagonist, D-Lys³ GHRP-6 (Figure 4). It was totally a new line of evidence to show how drinking H₂ water protect dopaminergic neurons; stimulation of β₁-adrenergic receptor in the stomach, releasing ghrelin into the blood, activating ghrelin receptors, and subsequently leading to inhibit dopaminergic neuronal death (Figure 5).

Clinical Assessment

In clinical study, either drinking H₂ water or infusion of H₂-rich saline is reported to be effective and the significance is noteworthy. For example, effects of H₂ water on antioxidant status of subjects was tested as an open-labeled pilot study in metabolic syndrome [48,49]. Also, effects of H₂ water on quality of life (QOL) of patients treated with radiotherapy for liver tumors [50] have been reported. According to the reports, consumption of H₂ water for 6 weeks reduced ROS in the blood and maintained blood oxidation potential. QOL scores

during radiotherapy were significantly improved in patients treated with H₂ water compared to patients receiving placebo water.

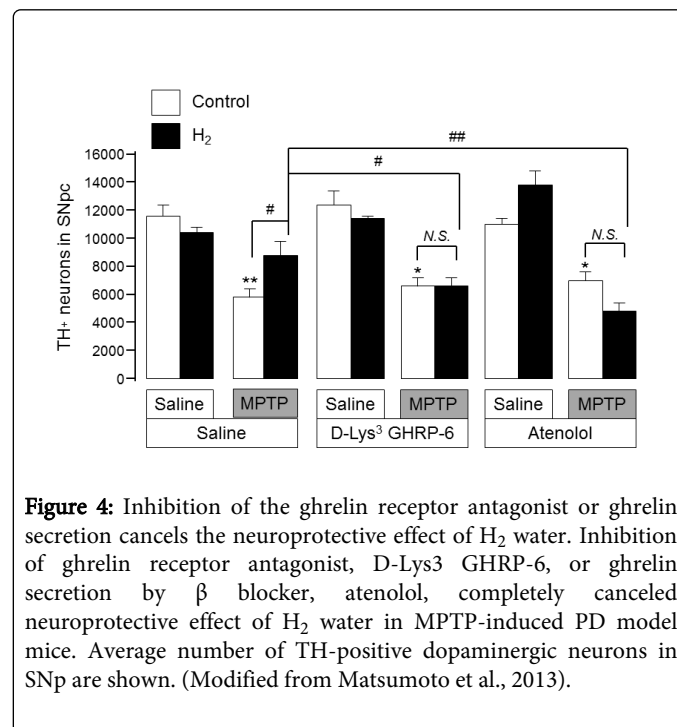
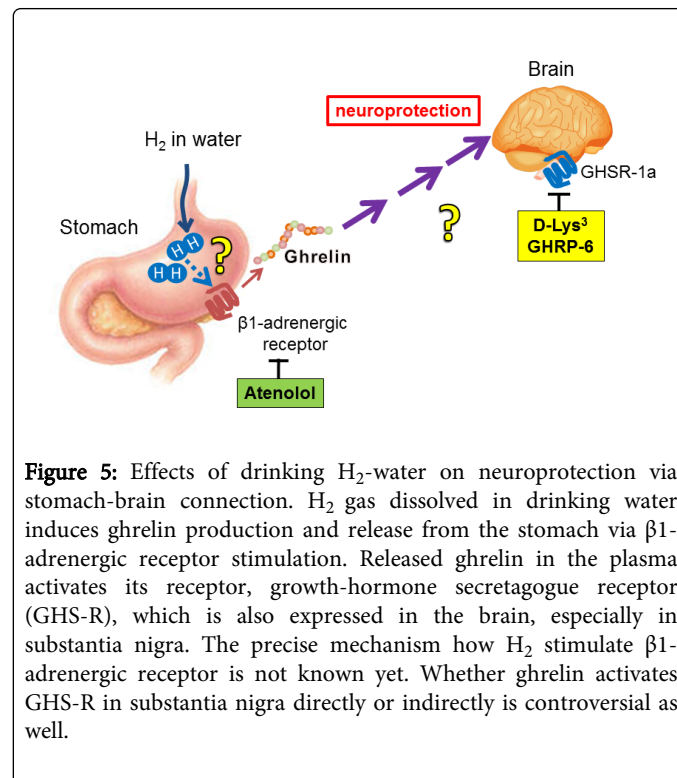


Figure 4: Inhibition of the ghrelin receptor antagonist or ghrelin secretion cancels the neuroprotective effect of H₂ water. Inhibition of ghrelin receptor antagonist, D-Lys³ GHRP-6, or ghrelin secretion by β blocker, atenolol, completely canceled neuroprotective effect of H₂ water in MPTP-induced PD model mice. Average number of TH-positive dopaminergic neurons in SNp are shown. (Modified from Matsumoto et al., 2013).



It is noteworthy that, in clinical study, infusion of H₂-rich saline also improved MRI indices during acute stage of brainstem infarction [51] and peritoneal deterioration [52]. A couple of studies on hemodialysis with H₂-rich saline were also reported [52-54].

No.	Disease	Design	Pts	Amount (ml)	Conc.	Weeks	Reference
1	Parkinson's disease	Double-blind RCT	19	1000	1.6	56	Yoritaka, et al., [55]
2a	Myopathies	Open label trial	14	1000	1	12	Ito, et al., [57]
2b	Myopathies	Double-blind crossover RCT	22	500	1	8	Ito, et al., [57]
3a	Diabetes mellitus type 2	Double-blind crossover RCT	30	900	1.2	8	Kajiyama, et al., [58]
3b	Glucose intolerance	Double-blind crossover RCT	6	900	1.2	8	Kajiyama, et al., [58]
4a	Metabolic syndrome	Open label trial	20	1500-2000	1.2	8	Nakao, et al., [48]
4b	Metabolic syndrome	Open label trial	20	900-1000	0.20-0.25	10	Song, et al., [49]
5a	Hemodialysis	Open label trial	8	hemodialysis	0.048	4	Nakayama, et al., [54]
5b	Hemodialysis	Open label trial	21	hemodialysis	0.21	26	Nakayama, et al., [53]
6	Radiation side effects	RCT but not blinded	49	1500-2000	1.1-1.3	6	Kang, et al., [50]
7	Brain stem infarction	Open label trial	34	drip infusion	1.6	1	Ono, et al., [51]
8	Muscle fatigue	Double-blind crossover RCT	10	1500	1.2	Once	Aoki, et al., [59]
9	Rheumatoid arthritis	Open label trial	20	530	5-Apr	4	Ishibashi, et al., [22]
10	Interstitial cystitis/painful bladder syndrome	Double-blind RCT	30	600	1.2	8	Matsumoto, et al., [56]
11	Peritoneal deterioration		6	Peritoneal dialysis	1.2		Terawaki, et al., [52]

RCT: Randomized Controlled Trial; Pts: Number of Patients; Conc., hydrogen concentration (ppm).

Table 1: Human diseases and states tested for the effects of molecular hydrogen.

A small-scale placebo-controlled, randomized, double-blind, parallel-group clinical trial has been recently reported for PD [55]. Participants took 1.000 mL/day of H₂ water or placebo water for 48 weeks. Total Unified Parkinson's Disease Rating Scale (UPDRS) scores in the H₂ water group (n=9) improved (median, -1.0; mean ± standard deviation, -5.7 ± 8.4), whereas UPDRS scores in the placebo group

(n=8) worsened (median, 4.5; mean ± standard deviation, 4.1 ± 9.2). Although the number of participants was minimal and the duration of the trial was extremely short for a neurodegenerative disease, the authors observed significant difference in the UPDRS scores (p< 0.05). A medium-scale multi-center clinical trial is currently in progress to our knowledge.

The effects of molecular H₂ have been tested in ten other human diseases or states (Table 1). Among the eleven human diseases or states, molecular H₂ showed mild to prominent effects in nine, and had no effect on interstitial cystitis/painful bladder syndrome [56]. Double-blind randomized placebo-controlled trials have been performed in five diseases or states: PD [55], mitochondrial and inflammatory myopathies [57], diabetes mellitus type 2/glucose intolerance [58], muscle fatigue in young soccer players [59], and interstitial cystitis/painful bladder syndrome [56]. As placebo effects potentially obscure the true benefit of molecular H₂, controlled studies are required for all the diseases.

Conclusion and Discussion

Molecular hydrogen (H₂ gas) shows protective and anti-inflammatory effects with multiple mechanisms. It has acute and chronic effects. Acute effects may be due to mainly anti-oxidant and a weak ROS-scavenger. Chronic effects need several days to develop and last several days even without actual presence of H₂. Though the molecular mechanisms on chronic effect of H₂ are not yet fully investigated, H₂ may serve as a beneficial gas and many evidences observed so far raise intriguing therapeutic and preventing options in the future.

Acknowledgments

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