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BRIEF

Molecular hydrogen may activate the transcription factor Nrf2 to alleviate oxidative stress through the hydrogen-targeted porphyrin

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Abstract

Oxidative stress is one of the major causes of most age-dependent neurodegenerative disorders. Neurons accumulate oxidative damage over time due to post-mitotic cells. Thus, modulation of oxidative stress is essential to overcome these disorders. Molecular hydrogen (H_2) has great potential for treating various diseases and improving quality of life by exerting multiple functions including anti-oxidation, anti-inflammation, and energy metabolism promotion. Among these functions, H_2 activates a transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) to enhance the transcription of transcribe a broad range of anti-stress enzymes, including antioxidant enzymes. There was an elusive contradiction between H_2 and Nrf2 because Nrf2 is activated in response to oxidative stress, whereas H_2 has a reducing potential. The target molecule for H_2 has recently been identified as the oxidized form of Fe-porphyrin conjugated with the -OH group (PrP-Fe(III)-OH). As the initial step, the hydroxyl radical (•OH) oxidizes heme (PrP-Fe(II)) to form PrP-Fe(III)-OH. Then, H_2 reacts with PrP-Fe(III)-OH to produce PrP-Fe(III)-H and H_2O. In turn, Fe(III) with H has the potential to act as an electrophile to oxidize Kelch-like ECH-associated protein 1 (Keap1), resulting in activating Nrf2. Thus, when the original highly damaging electrophilicity of •OH is buffered by H_2 and its target porphyrin, the electrophilicity provided by •OH can indirectly activate Nrf2 to reduce oxidative stress. Even without lowering the dosage, the effect of alleviated potent is considered to be hormesis-like. This "Therapeutic Brief" propose that the alleviated oxidative potent of •OH functions to activate Nrf2 as hormesis-like.

Keywords: Hematin, hydroxyl radical, molecular hydrogen, Nrf2, oxidative stress, porphyrin

Introduction

Molecular hydrogen (dihydrogen; H_2) is an inert molecule in the absence of a catalyst. It has long been believed that H_2 has no biological function in mammalian cells because mammals lack the genes encoding hydrogenases that catalyze reactions involving H_2 [1, 2]. In 2007, this concept was overturned by publishing the article entitled "Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals" [3]. This paper served as a trigger for the initiation of a new field of "hydrogen medicine and agriculture" [4, 5]. Subsequently, in addition to its antioxidant action, H_2 has been revealed to exert multiple functions such as anti-inflammatory, anti-allergic, anti-cell death, and metabolic stimulating effects by modulating various intracellular signal transductions [5, 6]. H_2 has no adverse effects, leading to extensive clinical studies for various diseases [5, 6]. In addition, H_2 not only improves patients with various diseases, but also supports the quality of life (QOL) of healthy people in various fields such as healthcare, sports, and beauty [5]. In 2014, the US Food Drug Administration (FDA) approved H_2 as generally recognized as safe (GRAS), allowing hydrogen-infused water to be marketed as a drink. In 2016, H_2 gas

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inhalation therapy was approved by the Japanese government as an advanced treatment for post-cardiac arrest syndrome [7]. Furthermore, H_2 is beneficial not only to animals and humans but also to higher plants. Therefore, H_2 can have a strong impact on agriculture [8].

However, the molecular mechanism by which H_2 exerts multiple functions remained unclear. The current Therapeutic Brief will discuss and propose a molecular mechanism by which H_2 with the hydrogen-targeted porphyrin activates the transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) to alleviate oxidative stress, suggesting a hormesis-like effect.

H_2 selectively reacts with hydroxyl radicals in living cells

Oxidative stress is derived by excessive generation of reactive oxygen species (ROS) such as superoxide anion radical ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), nitric oxide (NO), and hydroxyl radical (•OH) [9-10]. As neurons are post-mitotic cells, neurons accumulate oxidative damage over many years. However, ROS such as H_2O_2 and $\bullet O_2^-$ and NO play important physiological roles in signaling cascades and biological processes such as cell proliferation, differentiation, apoptosis, and immunomodulation [11-14], and thus, excessive antioxidant intake is not beneficial and induces mortality as published [15, 16].

Molecular hydrogen (H_2) selectively reduces highly toxic ROS, •OH and peroxynitrite ($ONOO^-$), but neither $\bullet O_2^-$, H_2O_2, nor NO [3]. In cell culture experiments, H_2 decreased the fluorescence signal of 3'-p-(hydroxyphenyl) fluorescein (HPF) when oxidative stress was induced in various ways [3]. HPF is an intracellular marker for •OH [17, 18]. Decrease in this fluorescent signal by H_2 was not only observed in cultured cells, but also in various tissues as shown in testicular radioprotection [19], hematopoietic stem cell damage by total body irradiation [20], and hyperoxia in cultured cells [21], lung hypoxia/reoxygenation [22], retinal ischemia-reperfusion [23], and retinal sonication [24].

H_2 can be infused into water (hydrogen water) up to a maximum of 0.8 mM at atmospheric pressure. After drinking H_2 water or inhaling H_2 gas, measuring the H_2 content revealed that H_2 is consumed in the human body [25, 26]. Deuterium gas (D2) was used in rats as a metabolic tracer to monitor D2 oxidation, indicating that molecular hydrogen is indeed oxidized *in vivo* [27].

Thus, H_2 was confirmed to decrease cellular •OH in a variety of ways across cell types and tissues although •OH is the most oxidative molecule to damage the cell components in a chaotic manner [10].

By the way, in homogeneous aqueous kinetics, the reaction rate of •OH with H_2 is much slower (the kinetic rate is 0.35×10^{-8} $M^{-1}s^{-1}$) than those with other antioxidants [28]. For example, •OH reacts with glucose and glutathione with kinetic rates (15×10^{-8} $M^{-1}s^{-1}$), and (230×10^{-8} $M^{-1}s^{-1}$), respectively [29]. The other biomolecules also react with •OH much faster than H_2. The contradiction between homogeneous aqueous solutions and living organisms has been debated for a long time.

Although H_2 cannot react with most molecules without a metal catalyst, effective amounts of metals such as Cu, Fe, Ni, and Pt are unlikely to be present in living cells. In addition, there is no report indicating the discovery of an organo-catalyst for H_2. Despite extensive worldwide research, it was hard to discover a catalyst that facilitates the reaction of H_2 with •OH. An H_2-target molecule as described below has recently been identified, providing a clue to explain the underlying contradiction of H_2.

Aging is associated with an increased incidence of neurodegenerative diseases

Aging is associated with an increased incidence of neurodegenerative disorders. This is because neurons accumulate oxidative damage due to post-mitotic cells over a long period [30]. Oxidative stress is one of the leading causes of most neurodegenerative disorders [31, 32]. Several animal studies indicate that H_2-treatment is potentially applicable to alleviate neurodegenerative disorders and improve the quality of life in the elderly [33-37]. Thus, H_2 is expected to ameliorate aging-related neurodegeneration. In particular, overcoming Alzheimer's disease (AD) is one of the most important challenges in the world's aging society [38].

It has been shown that drinking H_2 water reduces oxidative stress and ameliorated cognitive deficits in AD model mice [39]. Subsequently, a randomized, placebo-controlled, double-blind clinical trial was conducted on subjects with mild cognitive impairment (MCI), who drank 0.6 mM H_2 water (approximately 300 ml per day) for 1 year [39]. A sub-analysis showed that subjects with the apolipoprotein E (APOE4) genotype, a well-known genetic factor for AD [40, 41], were significantly improved. Improvement was assessed by the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog), one of the most reliable ways to assess cognition [42, 43].

H_2 inhalation has been applied in several clinical areas [5, 7, 44-46]. The most important feature of H_2 gas inhalation therapy is that it is non-cytotoxic and safe for humans, as approved in Phase I clinical trial [47].

A patient with severe Alzheimer's continued to inhale 3% hydrogen gas twice for one hour a day for two years. Diffusion tensor imaging (DTI) [48, 49] then visualized the activation of neurons of the patient, and urinary and fecal incontinence was improved [38]. This case report is of value even for a single case, as it is commonly understood that patients with severe AD are irreversible [38].

H_2 activates Nrf2 to function to reduce oxidative stress

Nrf2 transcribes the genes encoding several antioxidant enzymes to protect cells against oxidative stress [50-51]. Moreover, Nrf2 contributes not only to the reduction of

oxidative stress, but also to widespread fields, including toxicology [52], oncology [53], inflammation [54], ischemia stroke [55], and the aging process [56]. Nrf2's targets are the genes encoding NAD (P) H quinone oxidoreductase 1 (Nqo1), thioredoxin, reductase 1 (TXNRD1), heme oxygenase-1 (HO-1), glutathione S-transferase (GST), and so on [50-51]. Nrf2 is maintained in an inactive form in the cytosol when it forms a complex with the Kelch-like ECH-associated protein 1 (Keap1). Upon oxidation of the essential cysteine residues of Keap1 by electrophiles, Nrf2 is released from Keap1 and then translocated into the nucleus, enabling the transcriptions [50-51].

H_2 can induce the activity of Nrf2, as shown in many publications. In Nrf2 knockout mice, the effects of H_2 were at least partially attenuated, in protecting various cells and tissues in response to various stressors [57]. These findings are consistent with subsequent publications that Nrf2-activation is one of the antioxidant effects of H_2 [58-69]. Therefore, it is concluded that the activation of Nrf2 is involved in one of the H_2 functions.

As a molecular mechanism, it is unlikely that H_2 directly influences Nrf2. H_2 must indirectly activate Nrf2 through multiple steps. One idea was proposed that H_2 enhances mitochondrial respiratory activity to generate excess ROS, which in turn oxidizes intracellular Keap1 to release Nrf2 [70]. Alternatively, H_2 opens mitochondria-(ATP) K^+ channels [71, 72] to induce ROS [73]. However, although there is no doubt that H_2 activates Nrf2, there is no direct evidence that mitochondria-derived ROS can oxidize the residues of cytosolic Keap1. Moreover, an elusive contradiction exists between Nrf2 activation and H_2; activation of Nrf2 requires Keap1 oxidation, whereas H_2 has a reducing potential.

Target discovery of hydrogen molecules

A break-through paper entitled "Fe-porphyrins: redox-related biosensors of molecular hydrogen" has recently been published [74], showing that the molecular target/biosensor for H_2 is the oxidized form of Fe-porphyrins (designate "hematin"). This paper has shown the discovery that addresses the fundamental questions about the mechanisms in which H_2 is involved.

Hematin is an oxidized form of Fe(III)-containing porphyrin (PrP) converted from Fe(II)-containing porphyrin (heme) [75, 76]. This breakthrough paper showed a novel reaction showing that H_2 replaces the hydroxy group (-OH) conjugated to hematin Fe(III) with the hydrogen group (-H). The atom H of this -H group should behave as a hydride (H^-) and, due to its high reactivity, •OH was rapidly converted to H_2O by this catalyst (Figure 1).

Thus, heme (PrP-Fe(II)) has been shown to act as a catalyst for the following reaction (Figure 1).

(1) PrP-Fe(II) + •OH → PrP-Fe(III)-OH

(2) PrP-Fe(III)-OH + H_2 → PrP-Fe(III)-H + H_2O

(3) PrP-Fe(III)-H + •OH → PrP-Fe(II) + H_2O

The overall equation (4) indicates that heme (PrP-Fe(II)) catalyzed the following reactions:

(4) 2 •OH + H_2 → $2H_2O$

As noted above, the unresolved discrepancy between aqueous and live-cell reactions can be explained by the catalytic reaction according to the above equations (2) and (3).

At the same time, H_2 can reduce the oxidized porphyrin with Fe(III) to restore heme, the reduced form of Fe(II).

Proposal of a mechanism to elucidate the mechanism by which reducing H_2 activates Nrf2

Porphyrins are distributed everywhere inside and outside the living cells in the body. Heme is present in hemoglobin in the blood and myoglobin in muscles and is responsible

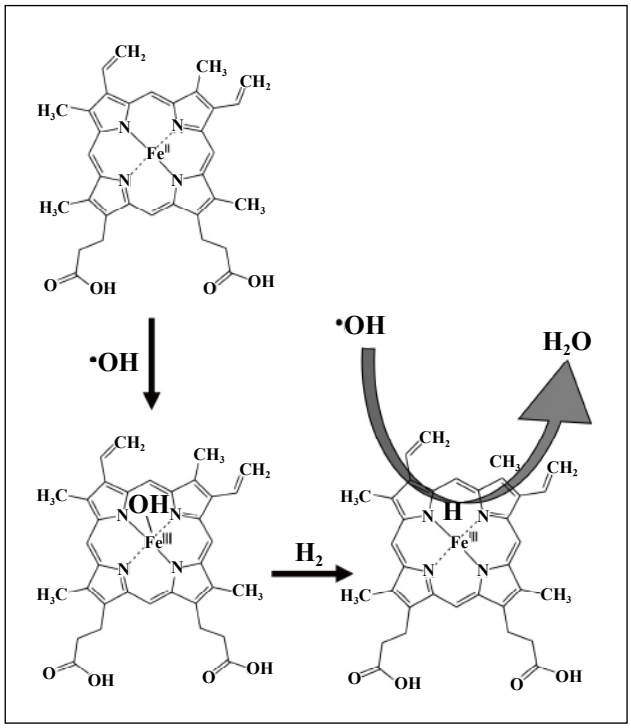

Figure 1. Fe-porphyrin catalyzes the reaction of H_2 with the hydroxyl radical.

(Equation 1) PrP-Fe(II) + •OH → PrP-Fe(III)-OH

(Equation 2) PrP-Fe(III)-OH + H_2 → PrP-Fe(III)-H + H_2O

(Equation 3) PrP-Fe(III)-H + •OH → PrP-Fe(II) + H_2O

The formal name of Hematin PrP-Fe(III)-OH is ferriprotoporphyrin IX hydroxide.

for delivering molecular oxygen (O_2) throughout the body [77]. Thus, heme is frequently exposed to O_2 or H_2O_2, and thus, Fe(II) of heme can frequently catalyze the formation of •OH by the Fenton reaction or its mimic reactions [78-80]. Porphyrins are located as cytochromes in the electron transport chain of the mitochondrial inner membrane, and play a role in electron transport by converting Fe(II) to/from Fe(III) [81]. In the intracellular cytosol, the antioxidant enzymes such as catalase [82] and peroxidase [83], P450 [84], and nitric oxide (NO) synthase [85] have porphyrins as an essential component [86]. These porphyrins

with Fe(II)/(III) act as mediators of redox reactions and are subject to oxidative stress.

Hematin (PrP-Fe(III)-OH) is converted from hemin (PrP-Fe(III)-Cl) [87, 88]. Notably, hemin (PrP-Fe(III)-Cl) activate Nrf2 [89-91] (Figure 2). The Fe(III) of hemin probably functions as an electrophile, oxidizing the residues of Keap1 and activating Nrf2. The electronegativities of H and Cl are 2.2 and 3.16, respectively. Thus, Fe(III) containing H should be more electrophilic than Fe(III) containing Cl, and may be able to oxidize Keap1 more efficiently according to the equation of PrP-Fe(III)-H + e^- →PrP-Fe(II) + 1/2 H_2 (Figure 2).

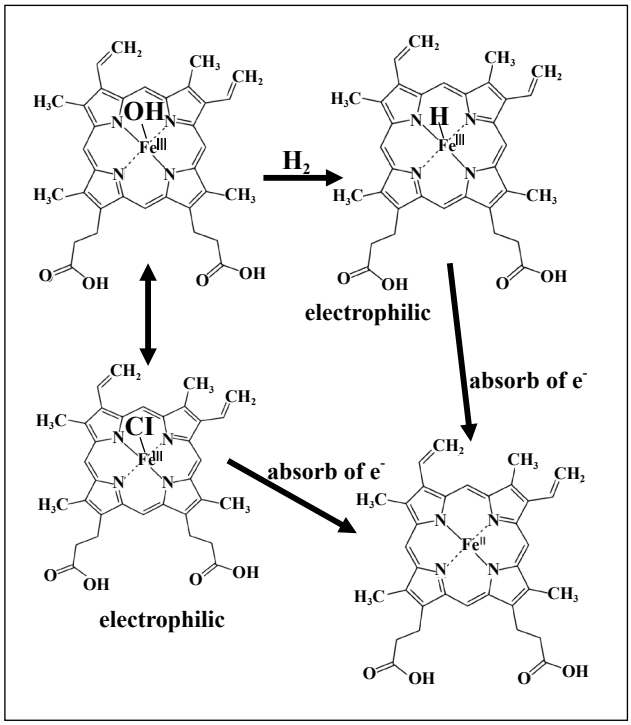

Figure 2. Fe(III) in hydride hematin can serve as an electrophile to oxidize the residues of Keap1.

Hematin and hemin can mutually be converted, and hemin is known to activate Nrf2 by oxidizing Keap1.

Fe(III) of hemin is electrophilic to activate Nrf2. Fe(III)-H may be more electrophilic than Fe(III)-Cl to efficiently oxidize Keap1, resulting in activating Nrf2.

The formal name of Hemin PrP-Fe(III) is ferriprotoporphyrin IX chloride.

As mentioned in the above equation (1) PrP-Fe(II) + •OH → PrP-Fe(III)-OH, hematin (PrP-Fe(III)-OH) was originally formed by oxidizing heme (PrP-Fe(II)) by •OH. •OH is the most oxidative molecule to damage biomolecules indiscriminately [10], but the strong electrophilicity of •OH can be buffered in the presence of H_2 and Fe-PrP, and resultant electrophilicity in Fe-PrP can contribute to activating Nrf2, resulting in reducing oxidative stress.

Hormesis is a term used by toxicologists to refer to a biphasic dose response to an environmental agent characterized by a low-dose stimulation or beneficial effect and a high-dose inhibitory or toxic effect [92] or defined as the paradoxical beneficial effects of low-dose stressors, which can be better defined as the biphasic dose-effect or time-effect relationship for any substance [93].

•OH is the most oxidative molecule that caused damage to biomolecules [10], but, the strong electrophilicity of •OH can be alleviated through stepwise reactions in the presence of H_2 and porphyrin.

Lowering the concentration of a toxic substance is reducing its toxicity. It is proposed that even without lowering the dosage, the effect of alleviated strong potent is considered to be hormesis-like.

Once the original strong electrophilicity of •OH is transferred to PrP-Fe(III)-OH and PrP-Fe(III)-H, it is possible that the alleviated oxidative potent contributes to the activation of Nrf2 as a hormesis-like effect.

The current proposal needs to be examined in more detail in the future.

Conclusion

H_2 acts as a therapeutic antioxidant [3] and activates Nrf2, which transcribes antioxidant enzymes to reduce oxidative stress and protected cells against various stresses. There was an unresolved contradiction between H_2's reductive property and Nrf2's requirement of oxidative stress for its activation. The target molecule for H_2 has recently been identified as the oxidized form of Fe-porphyrin conjugated with the OH group (PrP-Fe(III)-OH) [74]. H_2 and the H_2-targeting porphyrin can buffer the highly oxidizing electrophilicity of •OH. When the original •OH's oxidative and harmful electrophilicity is alleviated, the resultant electrophilic potent may contribute to the activation of Nrf2 as a hormesis-like effect.

Declarations

Authors' contributions: The author contributed solely to the article.

Availability of data and materials: Not applicable.

Conflicts of interest: The author is the director of H_2 WATER JAPAN, Inc., (Tokyo, Japan) and H_2 Global Group (Ostrava, Czech Republic), companies involved in H_2.

Ethical approval and informed consent statement: Not applicable.

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