Chapter 3
Prospects of Hydrogen Medicine Based on Its Protective Effects on Mitochondrial Function

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Abstract Mitochondria originated from aerobic bacteria in endosymbiosis. Through this symbiosis, eukaryotes acquired an efficient energy-producing system, but at the cost of exposure to oxidative stress from reactive oxygen species (ROS). Molecular hydrogen (H_2) was recently identified as an antioxidant that selectively reduces ROS, such as hydroxyl radicals and peroxynitrite, which are strong oxidants, and its clinical applications are progressing. This paper investigated the efficacy of H_2 on experimental models and several human chronic inflammatory diseases and demonstrated that its exerted effects via the protection of mitochondrial function. H_2 protection may be exerted by regulation of mitochondrial ROS. Since mitochondrial dysfunction has been detected in many common diseases, such as metabolic and neurodegenerative diseases, the development of technologies and substances that protect or activate mitochondrial function will be necessary for the future of

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medicine. H_2 may be positioned as a candidate in future medicine due to its effects on mitochondrial function.

Keywords Molecular hydrogen · Reactive oxygen species · Oxidative stress · Mitochondria · Inflammatory disease · Post-COVID-19 · ME/CFS · Future medicine

Introduction

Eukaryotes, which emerged approximately 2 billion years ago when archaea engulfed the aerobic bacteria, *proteobacteria*, acquired an efficient energy-producing system, but at the cost of exposure to oxidative stress caused by reactive oxygen species (ROS) produced in mitochondria [1]. The antioxidant enzymes are ineffective against the production of ROS, such as hydroxyl radicals (·OH) and peroxynitrite ($ONOO^-$), which are very strong oxidants [2, 3].

Molecular hydrogen (H_2) was recently identified as an antioxidant that directly reduces ·OH and $ONOO^-$ [4]. H_2 also exerts indirect antioxidant, anti-inflammatory, and anti-apoptotic effects by regulating gene expression [5–7]. Other indirect mechanisms by which H_2 exerts its effects have been reported, such as nuclear factor erythroid-related factor 2 (Nrf-2) and various signaling pathways in cells [8–10]. The total number of studies on the biological effects of H_2 now exceeds 1600 [11]. Among them, the total number of studies on human clinical trials is more than 120. Since no side effects have been observed with H_2 in human clinical studies, various clinical studies are underway to investigate its ameliorative effects on various pathological conditions [11].

It is considered that protective effects on mitochondrial function may be involved in the efficacy of H_2 against oxidative stress-associated diseases. In this study, we will examine the efficacy of H_2 in experimental disease models and in chronic inflammatory diseases including "sequelae" of coronavirus infection disease 2019 (COVID-19) and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Furthermore, we discuss the mechanisms by which H_2 ameliorates these diseases and its potential for future medicine.

Oxidative Stress Regulates Health and Disease

Production and Scavenging Systems of ROS

Oxygen accounts for approximately 20% of air and is essential for energy production in breathing organisms [2, 3, 12]. After oxygen is taken into the body, it is used by mitochondria in cells to produce adenosine triphosphate (ATP). However, 1–2% of

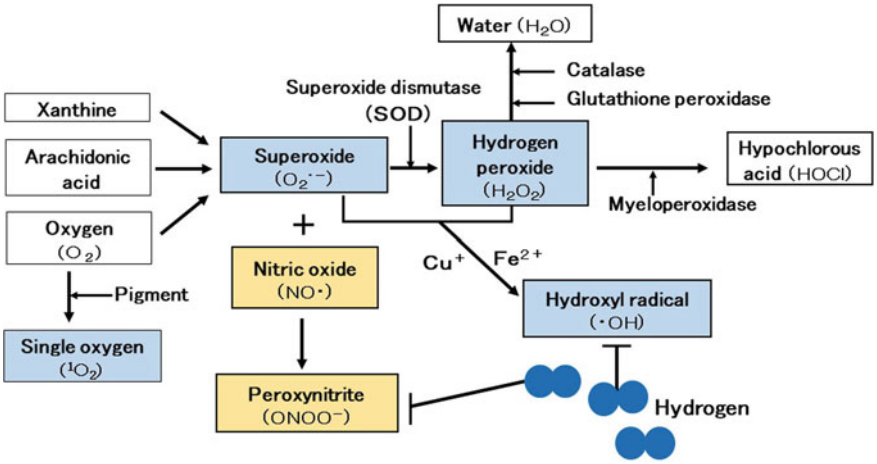

Fig. 3.1 *ROS production and scavenging systems.* Antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase, cannot scavenge ·OH or $ONOO^-$, which are strong oxidants. In contrast, H_2 selectively scavenges .OH and $ONOO^-$

consumed oxygen becomes ROS, which are strong oxidants in the body [2, 3, 12]. The human body is equipped with antioxidant enzymes as a defense mechanism to suppress the production of ROS. Antioxidant enzymes include superoxide dismutase (SOD), catalase, and glutathione peroxidase (Fig. 3.1).

There are four main types of ROS in the human organism: superoxide, hydrogen peroxide, ·OH, and singlet oxygen [2, 3, 12]. Singlet oxygen is produced by the reaction of oxygen with pigments in the body that function as sensitizers during exposure to ultraviolet radiation. When electrons leak from the mitochondrial respiratory chain and combine with oxygen, superoxide is formed [12]. In addition, superoxide is produced not only by oxygen and xanthine oxidase using xanthine as a substrate, but also by the arachidonic acid cascade in vascular endothelial cells. Superoxide is a relatively reactive substance but is decomposed by SOD to hydrogen peroxide [12]. Hydrogen peroxide is then decomposed into water and oxygen by catalase and glutathione peroxidase and detoxified in the body [12] (Fig. 3.1).

The function of antioxidant enzymes and the body's defense against ROS decline with age [2, 3]. Furthermore, when ROS are produced in large amounts due to excessive exercise, mental and physical stress, smoking, drinking, exposure to ultraviolet light and radiation, and air pollution, the balance between ROS production and scavenging systems is disrupted, resulting in the emergence of ROS that exceed the defenses of antioxidant enzymes and, ultimately, cell and gene damage [2, 3].

When the balance between oxidation and anti-oxidation is disrupted, superoxide and hydrogen peroxide produce ·OH, which is a very strong oxidant, using iron and copper ions as catalysts [2, 3, 12]. ·OH is produced by other biological reactions and also when water, a biological substance, is exposed to radiation [2, 3, 12]. On the other hand, nitric oxide produces $ONOO^-$, another very strong oxidant (Fig. 3.1).

Although ·OH is present in the body for only a fraction of a millionth of a second, it exhibits an oxidizing power that is 100-fold stronger than that of superoxide during that time [13].

Therefore, when ·OH and $ONOO^-$ are produced, they react with nucleic acids, lipids, and proteins in the membranes and tissues of living organisms, causing oxidative damage [2, 3]. They also oxidize DNA, which controls genetic information. However, superoxide and hydrogen peroxide do not have sufficient oxidizing power to directly oxidize DNA. The oxidation of DNA leads to genetic damage, which, in turn, induces lifestyle-related diseases, such as cancer [2, 3, 12]. Antioxidant enzymes cannot detoxify ·OH or $ONOO^-$. In contrast, H_2 selectively scavenges large amounts of ·OH and $ONOO^-$ and converts them to water (Fig. 3.1). For example, the chemical reaction equation between H_2 and ·OH is as follows:

$$\cdot OH + H_2 \rightarrow H \cdot + H_2O$$

ROS-Induced Oxidative Eustress and Distress

Diseases related to ROS are those that originate in many organs and tissues in the body, including the brain, nerves, eyes, nose, teeth, the respiratory, circulatory, digestive, urinary, hematological, and endocrine systems, skin, and supporting tissues [14]. Therefore, ROS have been implicated in the development of most diseases.

When the relationship between the generation of superoxide, a cause of oxidative stress, and life span was investigated, a negative correlation was observed, with organisms that generated more ROS having a shorter life span [15]. In addition, when the relationship between SOD and the life span of an organism (in terms of total lifetime energy) was examined, a correlation was also observed such that organisms with higher levels of this SOD activity had a longer life span [16].

ROS have not only harmful, but also beneficial aspects for organisms. Superoxide and hydrogen peroxide have been shown to exert cytotoxic effects at high concentrations, but function as molecules in signal transduction and play important roles in apoptosis, cell proliferation, and cell differentiation at low concentrations [2, 3]. In addition, hydrogen peroxide at high concentrations is converted to hypochlorous acid by antioxidant enzymes and plays a role in the body's defense against bacterial attacks [2, 3]. Nitric oxide is important for signal transduction and vasodilation and is used as a medical gas. In addition, differences have been observed between oxidative eustress and distress. A large amount of ROS causes oxidative damage, while a small amount induces heme oxygenase, an antioxidant enzyme, through the activation of Nrf-2, which exerts protective effects in the body [17]. Small amounts of ROS also induce p53, a tumor suppressor gene [18].

Mitochondrial Dysfunction and Disease

Mitochondria produce more than 90% of intracellular energy and generate ATP by oxidative phosphorylation under aerobic conditions. These organelles are composed of a bilayer structure of inner and outer membranes with an intermembrane space in addition to a matrix within the inner membrane [19]. The inner membrane has a narrow crista on the inner side [19]. The five proteins of the mitochondrial respiratory chain complex, complexes I-V, assemble in the inner membrane crista for efficient ATP production [19]. Moreover, many mitochondrial ROS (mtROS), primarily generated from complexes I and III, are removed by the antioxidant system within mitochondria, which includes SOD2/MnSOD, catalase, glutathione peroxidase, and reduced glutathione. Therefore, efficient energy production is maintained by the electron transfer system of the mitochondrial inner membrane respiratory chain complex as well as oxidative phosphorylation by ATP synthase [20, 21].

However, abnormalities in mitochondrial function induce a decrease in ATP production and an increase in mtROS, which, in turn, contribute to the development of pathological conditions due to apoptotic signals, such as cytochrome c released from within damaged mitochondria [20]. Mitochondrial dysfunction without genetic abnormalities has been detected in common metabolic and neurodegenerative diseases. Mitochondrial dysfunction has also been implicated in the pathogenesis of diabetes, atherosclerosis, hypertension, Parkinson's disease, acute kidney disease, and amyotrophic lateral sclerosis (ALS) [20, 22].

Mitochondrial diseases are caused by abnormalities in various genes involved in mitochondrial function and structural maintenance, such as ATP synthesis, the transport of amino acids, lipids, and proteins, and oxidative stress removal within mitochondria [20, 23]. In mitochondrial diseases, clinical symptoms develop in tissues that require large amounts of energy, mainly muscles and nerves, due to impaired mitochondrial respiratory function, and lactic acidosis is also induced due to an increased dependence on energy from the glycolytic system [24].

Oxidative stress-induced impairments in the structure and function of mitochondria have been implicated in the development of various diseases; however, mild stress caused by H_2 may actually enhance resistance to the exacerbation of oxidative stress. Murakami et al. used cultured cells to investigate the effects of low concentrations of H_2 on mitochondria [25]. Their findings revealed that H_2 increased the mitochondrial membrane potential (MMP) and intracellular ATP levels [25]. A pretreatment with H_2 inhibited hydrogen peroxide-induced cell death, whereas a post-treatment did not. In addition, H_2-treated cells showed the up-regulated expression of antioxidant enzymes involved in the Nrf-2 pathway. These findings suggest that H_2 functions not only as a radical scavenger, but also as a mitohormetic effector [25].

Effects of H_2 on Mitochondrial Function

Effects in Various Experimental Disease Models

H_2 shows efficacy in various disease models in experimental animals or cultured cells via improvements in the structure and/or function of mitochondria. Therefore, this chapter provides an overview of the literature demonstrating the efficacy of H_2 in disease models of stroke, subarachnoid hemorrhage (SAH), ALS, Alzheimer's disease, myocardial injury, hypertension, sepsis, diabetic neuropathy, and liver injury as well as the underlying mechanisms (Table 3.1) [26–34].

Table 3.1 Summary of representative findings in experimental disease models using hydrogen (H_2)

Disease models	Type of H_2	Effects of H_2	Ref. No.
Cerebral infarction	HRS	HRS attenuated neuronal I/R injury by protecting mitochondrial function in rats	[26]
Subarachnoid hemorrhage	H_2 gas	H_2 gas attenuated neuronal pyroptosis in a rat model of SAH through the mitoK$_{ATP}$ signaling pathway	[27]
ALS	HRS	HRS delayed disease progression in a mouse model of ALS by reducing oxidative stress and maintaining mitochondrial function	[28]
Alzheimer's disease	HRW	HRW attenuated Aβ-induced cytotoxicity through the up-regulation of Sirt1-FoxO3a by a stimulation of AMPK in SK-N-MC cells	[29]
Myocardial injury	H_2 gas	H_2 gas reduced infarct sizes in canine hearts through the opening of mitoK$_{ATP}$ channels followed by the inhibition of mPTP	[30]
Hypertension	HRS	HRS reduced oxidative stress and attenuated left ventricular hypertrophy through the preservation of mitochondrial function in SHR	[31]
Sepsis	H_2 gas	H_2 gas alleviated sepsis-induced brain injury by improving mitochondrial biogenesis through the activation of PGC-α in mice	[32]
Diabetic peripheral neuropathy	HRS	HRS protected against diabetic peripheral neuropathy through the activation of mitoK$_{ATP}$ channels in rats	[33]
Liver injury	HRS	HRS protected against mitochondrial dysfunction and apoptosis in mice with obstructive jaundice	[34]

HRS: hydrogen-rich saline; I/R: ischemia/reperfusion; SAH: subarachnoid hemorrhage; mitoK$_{ATP}$: mitochondrial ATP-sensitive K^+; ALS: amyotrophic lateral sclerosis; Aβ: amyloid-β; Sirt1: sirtuin 1; FoxO3a: forkhead box protein O3a; AMPK: AMP-activated protein kinase; mPTP: mitochondrial permeability transition pores; HRW: hydrogen-rich water; SHR: spontaneously hypertensive rat; PGC-α: peroxisome proliferator-activated receptor gamma co-activator 1α

Cerebral Infarction

Cui et al. examined the effects of hydrogen-rich saline (HRS) in a rat brain ischemia/reperfusion (I/R) model and demonstrated that it significantly increased the number of viable neurons [26]. They also showed that HRS not only suppressed tissue damage, the degree of mitochondrial swelling, and the reduction of MMP, but also maintained the mitochondrial content of cytochrome c [26]. These findings suggest that HRS attenuates neuronal I/R damage in rats by protecting mitochondrial function.

SAH

Zhang et al. investigated the protective effects of H_2 gas against neuronal pyroptosis in a rat model of SAH and reported that the inhalation of H_2 gas significantly ameliorated brain edema, improved neurological function, and inhibited neuronal pyroptosis [27]. Furthermore, H_2 gas suppressed ROS production, the expression of interleukin (IL)-1β and IL-18, and the activation of p38 mitogen-activated protein kinase (p38 MAPK), and these inhibitory effects of H_2 were attenuated by the administration of sodium 5-hydroxydecanoate (5-HD), a mitochondrial ATP-sensitive K^+ (mitoK_{ATP}) channel inhibitor [27]. Collectively, these findings suggest that the neuroprotective effects of H_2 gas involve the mitoK_{ATP}/p38 MAPK signaling pathway.

ALS

Zhang et al. examined the effects of HRS in a mutant SOD1 G93A transgenic mouse model of ALS and reported that it significantly delayed the onset of disease and prolonged survival [28]. They also showed that HRS inhibited the mitochondrial release of apoptogenic factors and subsequent activation of downstream caspase-3 [28]. Furthermore, they demonstrated that HRS maintained mitochondrial function, restored complex I and IV activities, decreased mtROS production, and promoted mitochondrial ATP synthesis [28]. Based on these findings, they suggested that H_2 exerts neuroprotective effects against ALS by reducing oxidative stress and maintaining mitochondrial function.

Alzheimer's Disease

Lin et al. investigated the effects of H_2-rich water (HRW) on the cytotoxicity of human neuroblast SK-N-MC cells [29]. They demonstrated that HRW reduced excess ROS, inhibited oxidative damage, and suppressed Amyloid-β (Aβ)-induced cell death [29]. Furthermore, they showed that HRW stimulated AMP-activated protein kinase, which up-regulated the forkhead box protein O3a (FoxO3a) downstream antioxidant

response and reduced Aβ-induced mitochondrial potential loss and oxidative stress [29]. They also indicated the potential of HRW as an effective therapeutic agent to inhibit Aβ-induced neurotoxicity.

Myocardial Injury

Yoshida et al. examined the effects of H_2 gas in a myocardial I/R model in beagle dogs and found that it reduced myocardial infarct sizes; however, the administration of 5-HD or atractyloside, a mitochondrial permeability transition pore (mPTP) opener, nullified the effects of H_2 gas on infarct sizes [30]. These findings indicate that H_2 gas reduced infarct sizes in the canine heart by opening mitoK_{ATP} channels and inhibiting mPTP.

Hypertension

Yu et al. investigated the effects of the chronic administration of HRW on left ventricular hypertrophy in spontaneously hypertensive rats (SHR) [31]. The findings obtained showed that HRW suppressed the production of inflammatory cytokines and nuclear factor-κB (NF-κB) activation in the left ventricle [31]. Furthermore, HRW maintained mitochondrial function by restoring electron transport chain enzyme activity, inhibiting ROS production, and enhancing ATP production [31].

Sepsis

Xie et al. examined the effects of H_2 gas on mitochondrial function and biosynthesis as well as the associated regulatory mechanisms in mice with sepsis-associated encephalopathy (SAE) [32]. Their findings showed that H_2 gas prolonged survival and preserved cognitive function in SAE mice and increased MMP and ATP levels, parameters of mitochondrial function, as well as the expression of complex I activity [32]. They also demonstrated that H_2 gas up-regulated the expression of the mitochondrial biosynthesis parameters, peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and mitochondrial transcription factor A [32]. These findings indicate that H_2 gas alleviated sepsis-induced brain damage in mice by promoting mitochondrial biosynthesis through the activation of PGC-1α.

Diabetic Peripheral Neuropathy

Jiao et al. investigated the efficacy of HRS against diabetic peripheral neuropathy (DPN) in a streptozotocin-induced diabetic rat model [33]. HRS significantly suppressed the behavioral, biochemical, and molecular effects of DPN in rats [33]. In

addition, 5-HD partially attenuated the therapeutic effects of HRS [33]. These findings suggest that the mechanisms underlying the efficacy of HRS against DPN involve the suppression of oxidative stress, inflammation, and apoptosis via the activation of the mitoK$_{ATP}$ pathway.

Liver Injury

Liu et al. investigated the effects of HRS in a mouse model of obstructive jaundice and found that it significantly reduced mitochondrial swelling, cytochrome c release, and oxidative damage [34]. They also reported that HRS suppressed cellular B-cell/CLL lymphoma 2 (Bcl-2)-associated x (Bax) protein expression, caspase activity, and hepatocyte apoptosis, and alleviated mitochondrial morphological defects [34]. These findings indicate that HRS inhibited mitochondrial oxidative stress and dysfunction and suppressed mitochondria-mediated apoptosis.

Effects on Chronic Inflammation

Inflammation is induced by inflammatory cytokines released by innate immunity. The cascade leading to the release of inflammatory cytokines is very complex; however, pathogens, such as viruses and bacteria, substances produced when the body is damaged, and irritants in the environment function as signals when inflammation is induced [35]. These extracellular signals cause mitochondrial dysfunction and induce the excessive production of ROS [36]. Excessive mtROS production and the release of oxidized mitochondrial DNA (mtDNA) trigger the formation of the nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, a protein complex [37, 38]. The NLRP3 inflammasome then activates the proteolytic enzyme caspase 1, which releases mature, transformed inflammatory cytokines from immune cells, such as macrophages and neutrophils, resulting in inflammation [37, 38] (Fig. 3.2).

We conducted a literature review on the suppression of acute and chronic inflammation by H_2 [38]. Many studies reported that the suppression of mtROS production by H_2 was involved in the anti-inflammatory effects of H_2 [38]. However, these studies did not specify the ROS involved [39–41]. Among ROS, ·OH is very oxidative and causes not only nuclear DNA damage, but also mtDNA damage and induces cell death. H_2 is a substance with excellent permeability to mitochondria and selective scavenging ability of ·OH [4]. Therefore, we showed that the mechanism underlying the amelioration of chronic inflammation by H_2 may involve the reduction of ·OH by H_2 protecting mtDNA from oxidative damage, which then suppresses a series of signaling events from the activation of the NLRP3 inflammasome to the release of inflammatory cytokines [38]. In this review, we proposed that the suppression of ·OH production by H_2 may be a mechanism inhibiting subsequent inflammatory signaling [38] (Fig. 3.2).

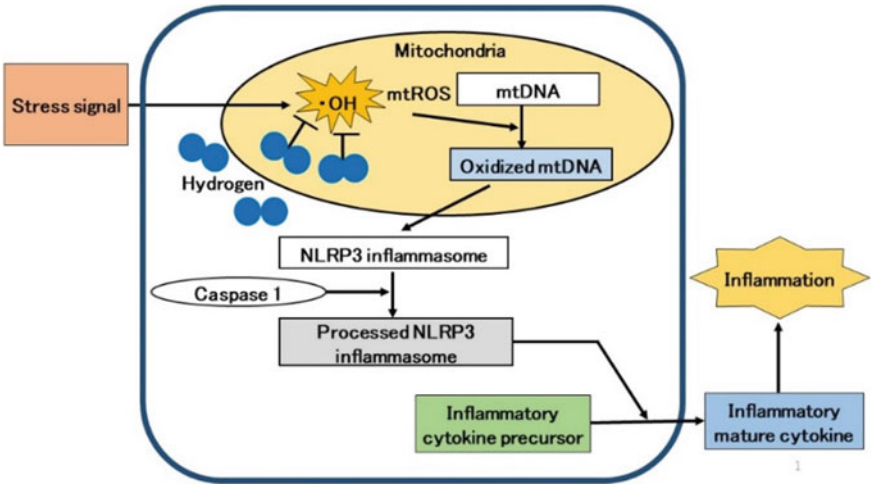

Fig. 3.2 *Involvement of mitochondria in the release of inflammatory cytokines.* H_2 inhibits the cascade from NLRP3 inflammasome activation to inflammatory cytokine release based on the inhibition of mtROS production

However, other researchers indicated that the downstream signaling pathways involved in cellular responses other than ROS are involved in the mechanisms responsible for the H_2-mediated suppression of inflammation [42–45].

Effects on "Sequelae" of COVID-19 and Chronic Fatigue

Guan et al. assessed the efficacy of inhalation therapy with a mixture of H_2 and oxygen gas in patients with COVID-19 in an open-label study [46]. They demonstrated the superiority of mixed H_2 and oxygen gas therapy over the control treatment for improvements in the severity of COVID-19, dyspnea, cough, chest tightness, chest pain, and oxygen saturation [46]. Although the inhalation of H_2 gas ameliorates the pneumonia symptoms of COVID-19, it is not only this infection that is problematic, but also the "sequelae" after contracting the infection [47, 48]. In other words, COVID-19 is a viral infection caused by SARS-CoV-2 that affects the respiratory, digestive, and vascular systems. Acute symptoms generally resolve within two to three weeks. However, for some patients, the recovery period is prolonged, and "sequelae" may persist for several months after the initial infection [47, 48]. These "sequelae" have been reported to include fatigue, dyspnea, myalgia, exercise intolerance, sleep disturbances, poor concentration, anxiety, fever, headache, malaise, and many other chronic symptoms [47, 48]. The "sequelae" of COVID-19 are referred to as post-COVID-19 or long-COVID-19.

Botek et al. recently reported the findings of a randomized, single-blind study on the effects of H_2 gas inhalation on physical and respiratory functions in acute post-COVID-19 patients [49]. They showed that H_2 gas inhalation more effectively attenuated clinical symptoms in acute post-COVID-19 patients than placebo gas because H_2 gas significantly improved physical function in gait tests and respiratory function in pulmonary function tests [49]. These findings indicate that H_2 gas inhalation exerts therapeutic effects not only on the pneumonia symptoms of COVID-19 patients, but also on the physical and respiratory functions of post-COVID-19 patients.

Post-COVID-19 may be the same as ME/CFS, which is characterized by severe fatigue lasting more than 6 months, extreme exhaustion after exertion, memory impairment, concentration problems, and sleep disturbances as the main symptoms [50]. Although the etiology of ME/CFS is unknown, mitochondrial dysfunction has been reported as one of its causes [51–54]. Since the symptoms of ME/CFS and post-COVID-19 are very similar, it has been argued, but not yet proven, that these diseases may be caused by the same mechanism [55].

Extensive efforts have been made to develop treatments and therapies for ME/CFS; however, the treatments and therapies that have been developed are symptomatic, not curative treatments [56]. We conducted a literature review and showed that H_2 ameliorated fatigue induced by acute or chronic exercise stress in experimental animals and healthy individuals, that fatigue was caused by mitochondrial dysfunction due to ROS, and that H_2 not only ameliorated acute and chronic fatigue by reducing ·OH, but also the pathogenesis of ME/CFS by reducing ·OH (Fig. 3.3) [57]. We also performed a case study on H_2 gas inhalation in four ME/CFS patients and found that it attenuated symptoms such as "brain fog", fatigue, the recovery time from exertion, headache, poor concentration, and sleep disturbances [58]. Although the efficacy of H_2 gas in many patients needs to be confirmed in future randomized controlled trials, the inhalation of H_2 gas will not only show efficacy for post-COVID-19, but also for ME/CFS.

Prospects for Future Medicine

We herein outlined the findings of previous studies showing that H_2 ameliorates diseases by protecting/improving the structure and/or function of mitochondria in various animal and cellular models [26–34]. Transmission electron microscopy revealed that H_2 exerts protective effects on the mitochondrial morphology [26, 34]. Biochemical and molecular evaluations of the protective effects of H_2 on mitochondrial function demonstrated that it suppressed ROS production and IL-1β and IL-18 expression and enhanced ATP production, MMP, and complex I activity [26–29, 31, 32, 34]. Regarding pharmacological evaluations of H_2, the administration of 5-HD, an inhibitor of mitoK_{ATP} channels, and atractyloside, an opener of mPTP, attenuated the effects of H_2, suggesting that H_2 exerts protective effects on mitochondrial function through the opening of mitoK_{ATP} channels and inhibition of mPTP [27, 30, 33]. Furthermore, H_2 inhibited the activities of caspase-3 and -9, suppressed the

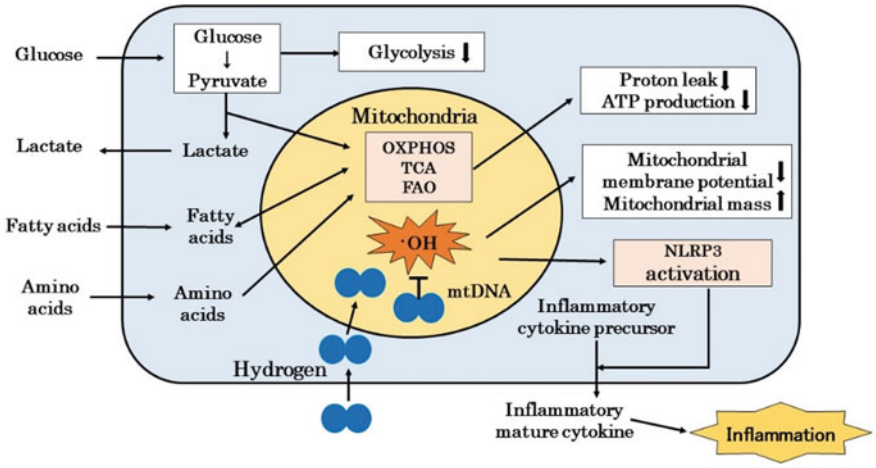

Fig. 3.3 *A possible mechanism by which H_2 ameliorates mitochondrial dysfunction in patients with ME/CFS*. The mitochondria of the patients show a reduced glycolytic capacity and abnormal metabolism. These mitochondria show decreased proton leakage, ATP production, and mitochondrial membrane potential and an increased mitochondrial mass. H_2 ameliorates mitochondrial dysfunction by scavenging ·OH and blocks the cascade from NLRP3 activation to the release of inflammatory cytokines. OXPHOS: oxidative phosphorylation, TCA: tricarboxylic acid cycle, FAO: fatty acid oxidation. (From [57])

release of cytochrome c and the expression of Bax, and increased the expression of Bcl-2, indicating that it protects mitochondrial function by inhibiting apoptotic cell death [34]. Collectively, these experimental findings showed that H_2 protects the mitochondrial morphology and/or function via the regulation of ROS production in the mitochondria.

ROS produced by mitochondrial dysfunction or failure cause various diseases, which originate in various organs and tissues of the body [14]. On the other hand, diseases involving chronic inflammation also originate from various organs and tissues of the body [14]. Therefore, while ROS are the cause of many diseases, chronic inflammation has also been implicated in their pathogenesis. We examined the mechanisms by which H_2 ameliorates chronic inflammation and found that they may involve protective effects on mitochondria based on the reduction of ·OH by H_2 [38]. We also hypothesized that the mechanisms by which H_2 ameliorates post-COVID-19 and ME/CFS may involve protective effects on mitochondria based on the reduction of ·OH by H_2 [57].

Modern medicine is characterized by viewing the human body as a collection of organs and subdividing the object of study from organs to cells, then to molecules, and finally to genes in order to identify the factors that most influence disease. However, many diseases are not caused by a single factor alone, but by multiple factors and a wide variety of mechanisms. H_2 is a substance that falls outside the scope of modern medicine because it has a wide range of effects on diverse diseases by acting on ROS and chronic inflammation, which are the root causes of disease [38].

One limitation of H_2 medicine is that because H_2 is used to treat a wide range of diseases, patients purchase and use H_2 water or H_2 gas inhalers independently without medical supervision, which may worsen medical conditions. Although many clinical studies have been conducted, research on doses and dosages for individual diseases is in the initial stages. Further basic and clinical research on individual diseases will be needed. In addition, a target molecule of H_2 was recently identified by Jin et al. [59]. An oxidized form of porphyrin catalyzes the reaction of H_2 with OH to reduce oxidative stress [59]. However, research into H_2 target molecules is still in its early stages, so further research is needed.

Conclusions

We herein reported the effects of H_2 on various experimental cellular and animal models and several human chronic inflammatory diseases including ME/CFS and post-COVID-19. We showed that it exerted effects via the protection of mitochondrial function due to its regulation of mtROS. In addition, we have shown that oxidative stress and chronic inflammation caused by mitochondrial dysfunction may cause various diseases. Since future medicine will require the development of technologies and substances that protect and activate mitochondrial function, H_2 may be positioned as a candidate in future medicine based on its effect on mitochondrial function.

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