Journal of the Neurological Sciences 396 (2019) 240–246

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Review Article

Hydrogen as a complementary therapy against ischemic stroke: A review of the evidence

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ARTICLE INFO

Keywords:
Ischemic stroke
Hydrogen therapy
Mechanism
Model
Clinical trial

ABSTRACT

Ischemic stroke is one of the most common sources of mortality in the world. Researchers have been trying to find a complementary therapy to treat ischemic stroke in order to improve its prognosis and expand the therapeutic window for reperfusion treatment. For this reason, many experimental and clinical trials studying the effects of hydrogen against ischemic stroke have been published. Hydrogen gas has been found to eliminate hydroxyl free radical and peroxynitrite anions as well as producing therapeutic effect in patients with ischemic stroke. Many studies have been published illustrating its anti-oxidative, anti-inflammatory and anti-apoptotic effects. The purpose of this article is to review the literature concerning treatment of cerebral I/R injury or ischemic stroke with hydrogen therapy. Specifically, we will examine the appropriate laboratory methods, mechanisms of hydrogen therapy, and outcomes of relevant clinical trials. We conclude this review with a discussion on future investigations of hydrogen therapy to treat ischemic stroke.

1. Introduction

Cerebral stroke is a severe medical condition occurring in the brain, induced by an occlusion or rupture of cerebral blood vessels. This condition can be differentiated into two types, ischemic stroke and hemorrhagic stroke. According to statistics published in 1999, among 795,000 stroke victims, 87% were affected by ischemic stroke [1]. Every three minutes and forty-five seconds, a patient dies of a stroke, with the age-adjusted mortality rate for stroke being 37.6 per 100,000 people [1].

Thus, the treatment of stroke, especially ischemic stroke, is a critical issue for all medical practitioners. Traditional therapy for ischemic stroke includes intravenous administration of recombinant tissue plasminogen activator (rt-PA) and surgical removal of the blood clot. Recently, endovascular thrombectomy provided a new reperfusion therapy for ischemic stroke. However, problems also remain, such as a narrow reperfusion time-window of just six hours and a poor prognosis [2]. Researchers have been trying to find new complementary therapies to improve the benefit of reperfusion therapy.

It has been proved that hydrogen has anti-oxidative, anti-inflammatory and anti-apoptotic effect. Ohsawa et al. first reported that molecular hydrogen reduced hydroxyl free radicals and peroxynitrite anions, which can induce necrosis, apoptosis, inflammation and dysfunction of brain, after ischemia and reperfusion (I/R) injury of the rat brain [3]. Since then, hydrogen has been proven to have protective effects towards intestinal and cardiac I/R injury [4,5]. Recently, several researches have shown that hydrogen can affect signal transduction pathways and gene expression, which has expanded the scale of

Abbreviations: rt-PA, recombinant tissue plasminogen activator; I/R, ischemia and reperfusion; MCAO, middle cerebral artery occlusion; CCAO, common carotid artery occlusion; 4-VO, four vessel occlusion; ICA, internal carotid artery; ROSC, internal carotid artery; OGD/R, oxygen glucose deprivation/reoxygenation; NS, neurological score; NDS, neurological deficit score; TH, therapeutic hypothermia; NSS, neurological severity score; HI, hypoxia ischemia; TTC, Triphenyl tetrazolium chloride; HE, Hematoxylin-eosin; NeuN, anti–neuronal nuclei antibody; 8-OH-DG, 8-hydroxy-2 deoxyguanosine; HNE, Hydroxynonenal; MDA, malondialdehyde; SOD, superoxidase; CAT, catalase; MnSOD, manganese superoxide dismutase; TGCI, Transient Global Cerebral Ischemia; RSG, rosiglitazone; 3-NT, 3-nitrotyrosine; Iba-1, ionized calcium binding adapter molecule 1; GFAP, glial fibrillary acidic protein; TNF-α, tumor necrosis factor-α; IL-6, interleukine-6; IL-1β, interleukine-1β; TGF-1β, transforming growth factor-1β; COX-2, cyclooxygenase; NF-κB, Nuclear factor κB; TUNEL, TdT-mediated dUTP Nick-End Labeling; RCT, randomized controlled trial; ADL, Activities of daily living; BI, Barthel Index; BRS, Brunnstrom Stage; mRS, modified Rankin Score; FIM, Functional Independence Measure; TTM, target thermal management

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https://doi.org/10.1016/j.jns.2018.11.004
Received 7 September 2018; Received in revised form 29 October 2018; Accepted 2 November 2018
Available online 05 November 2018

Table 1
This table summarized researches about therapeutic effects of hydrogen against cerebral I/R injury. Author names, publication dates, selection of models, ways of administration, parameters improved by hydrogen therapy were included in this table.

Number	Author	Year	Model	Way of administration	Parameters improved by hydrogen therapy					
					General condition	Tissue/cell damage	Oxidative biomarkers	Inflammatory biomarkers	Apoptotic biomarkers	Other biomarkers
1	Ohsawa	2007	In vitro: Antimycin A; OGD/R	Hydrogen dissolved in medium		Cell counting↑, MTT↑, LDH leakage↓	HPF↓, 8-OH-dG↓, HNE ↓, ONOO-↓			TMRM↑, ATP level↑
			In vivo: MCAO	Hydrogen inhalation	Neurological score ↓, Temperature ↑, Body weight ↑	Infarct volume(TTC, HE)↓	8-OH-dG↓, HNE↓	Iba-1↓, GFAP↓		
2	Cai	2009	In vivo: CCAO	Hydrogen saline injection	Postural Reflex Test↓, Locomotor activity↓, Body weight ↑, Escape latency from morris	Infarct volume(TTC)↓, Cell counting(Nissl)↑	MDA↓	Iba-1↓	TUNEL↓, Caspase-3↓	
3	Hugyecz	2011	In vivo: CCAO	Hydrogen inhalation				COX-2↓		nNOS↑, Connexin 30↓, Connexin 43↓
4	Ji	2011	In vivo: 4-VO	Hydrogen saline injection		Infarct volume(HE)↓, Cell counting(Nissl)↑	MDA↓	NF-κB↓, TNF-α↓, IL-6↓	Caspase-3↓	
5	Liu	2011	In vivo: MCAO	Hydrogen saline injection	Neurological scores ↓, Body weight↑	Infarct volume(TTC)↓, Cell counting(Nissl)↑	MDA↓, 8-OH-dG↓	IL-1β↓, TNF-α↓	TUNEL↓, Caspase-3↓, Bcl-2↑, Bax↓	Brain Water Content↓
6	Ge	2012	In vivo: bCCAO	Hydrogen inhalation	Escape latency from morris water maze↓	Infarct volume(HE)↓, Cell counting(HE)↑	8-isoPGF2α↓, MDA↓, SOD↑, CAT↑			
7	Hayashida	2012	In vivo: ROSC	Hydrogen inhalation	Survival rate↑, NDS↓			IL-6↓		
8	Li	2012	In vivo: MCAO	Hydrogen saline injection	Neurologic behavior scores↓	Infarct volume(TTC)↓	SOD↑, CAT↑, MDA↓, 8-isoPGF2α↓	TNF-α↓, HMGB1↓		
9	Nagatani	2012	In vivo: bCCAO	Hydrogen inhalation		Infarct volume(HE)↓, Cell counting(Nissl)↑	8-OH-dG↓, MDA↓			Brain water content↓, LC3↓, Albumin↓
10	Huang	2013	In vivo: ROSC	Hydrogen saline injection	Neurological scores↓, Survival Rate↑	Cell counting(Nissl)↑	8-OH-dG↓, MDA↓, SOD↑, CAT↑		TUNEL↓	
11	Zhai	2013	In vivo: MCAO	Lactulose oral intake	Neurological scores↓, Escape latency from morris water maze↓	Infarct volume(TTC)↓, Cell counting(Nissl)↑	SOD↑, MDA↓, 8-OH-dG ↓, 3-NT↓, Nrf2↑		TUNEL↓, Caspase-3↓	
12	Cui	2014	In vivo: 4-VO	Hydrogen saline injection		Infarcct volume(HE)↓				Ultrastructure of mitochondria↑, Mitochondrial membrane potential (MMP)↑, Cytochrome c↑
13	Hayashida	2014	In vivo: ROSC	Hydrogen inhalation	Survival rate↑, NDS↓, Y-Maze Test↑	Neuron counting(NeuN)↑, Axonal damage(MAP2)↓, Neuronal degeneration (FJC laberling)↓		Iba-1↓, GFAP↓		
14	Han	2015	In vivo: MCAO	Hydrogen saline injection	Neurological scores↓	Infarct volume(TTC)↓, Cell counting(DAPI/PI)↑				Hippocalcin↑, Parvalbumin↑, Intracellular Ca2 + ↑
15	Jin	2016	In vivo: MCAO	Hydrogen inhalation		Infarct volume(TTC)↓, Cell counting(Nissl, DAPI)↑	8-OHdG↓, 3-NT↓	IL-1β↓, IL-6↓, TNF-α↓	TUNEL↓, Caspase-3↓	
16	Li	2016	In vivo: 4-VO	Hydrogen saline injection	NSS↓	Infarct volume(HE)↓, Cell counting(HE)↑		TGF-β1↑, TNF-α↓, CD4 + CD25 + FOXP3 + T cell↑, NF-κB↓		miR-21↓, miR-210↓
17	Chen	2017	In vivo: ROSC	Hydrogen inhalation	NDS↓	Infarct volume(HE)↓				

(*continued on next page*)

Table 1 (*continued*)

Number	Author	Year	Model	Way of administration	Parameters improved by hydrogen therapy					
					General condition	Tissue/cell damage	Oxidative biomarkers	Inflammatory biomarkers	Apoptotic biomarkers	Other biomarkers
18	Wu	2018	In vitro: OGD/R	Hydrogen dissolved in medium		Cell viability(MTT)↓	ROS(DCFH-DA staining)↓		Annexin V/PI↓	MMP↑, LC3 and TOM20(mitophagy)↑, PINK1↑, Parkin↑

hydrogen study.

It is understood that reperfusion therapy of stroke patients will lead to I/R injury in brain. A large body of research has been published on protection of cerebral I/R injury and stroke mediated by hydrogen, illustrating the mechanism and effect of hydrogen from different aspects. In the following article, the experimental methods, and the mechanisms of hydrogen therapy, such as anti-oxidative, anti-inflammatory and anti-apoptotic effects, are summarized. Several clinical trials are also introduced in this article, which provide some ideas of trial designs regarding hydrogen treatment. Then we discuss the future of hydrogen therapy research.

2. The therapeutic effects of hydrogen on ischemic stroke or brain I/R injury in laboratory research

2.1. In vitro effects of hydrogen

2.1.1. In vitro models

There is not much in vitro research about the effects of hydrogen on I/R injury of the brain. Typical cellular research from Ohsawa et al. (Table, number 1) examined antimycin A, a mitochondrial respiratory complex III inhibitor. This compound was administrated to a cell culture medium to block the electron transport chain and create an environment of oxidative stress [3]. However, this model does not model ischemia of tissue, so it has not been widely utilized.

Oxygen glucose deprivation/ reoxygenation (OGD/R) is also a cellular model commonly used in experimentation [6]. According to the research of Wu et al. (Table, number 18), cells were exchanged into a glucose-free medium and were incubated in an environment of 94% N_2, 5% CO_2 and 1% O_2 for two hours. Then they were moved to a regular medium and were cultured with conditions consisting of 75% N_2, 20% O_2, and 5% CO_2 for reoxygenation. This method could better imitate the cellular conditions of ischemic stroke than the oxygen deprivation model, and so should be utilized more widely in future experiments.

2.1.2. Hydrogen attenuated cell damage under oxidative stress or OGD/R injury

The study by Ohsawa et al. (Table, number 1) showed that hydrogen gas ameliorated cell damage after oxidative damage due to antimycin A [3]. MTT assay was performed to measure the viability of cells, and the result was presented as optical density of nutrient solution at 490 nm. By the increase of antimycin A concentration, the viability of cells decreased. Hydrogen significantly improved the viability of cells in different concentrations of antimycin A (10 μg/ml and 30 μg/ml), which means it can protect cells from oxidative stress. Hydrogen also ameliorated cellular LDH leakage from damaged cells according to their research.

Wu et al. performed an OGD/R model to mimic ischemic condition of cells [6]. MTT was also used to estimate cell viability. According to their results, hydrogen improved the cell viability after OGD/R injury.

2.2. In vivo effects of hydrogen

2.2.1. Different methodologies for the administration of hydrogen

Inhalation is a practical strategy for the administration of hydrogen, in which animals receive different concentrations of hydrogen, such as 1% [3], 1.3% [7,8], 2% [3,9–11], 2.1% [12], 4% [3], and 66.7% [13] in combination with oxygen or other gases such as nitrogen and anesthetics.

Hydrogen-rich water is another popular administration method. According to Ji et al. (Table, number 4), such water was produced by dissolving hydrogen into physiologic saline under a pressure of 0.4 MPa for six hours [14]. Other researches also used similar procedures, but the pressure and time of aeration used were different [15–19]. Hydrogen-rich saline was then administrated intraperitoneally.

Interestingly, Zhai et al. (Table, number 11). described a new mode

of hydrogen administration by giving lactulose orally [20]. Lactulose can be bacterially fermented in the colon and produce molecular hydrogen gas. They also proved that this therapeutic effect could be abolished with the administration of antibiotics.

2.2.2. In vivo models

In order to investigate the therapeutic effect of hydrogen against the effects of ischemic stroke or cerebral I/R injury, a stable, practical and clinically relevant animal model needs to be established. Several animal injury models have been used in previous studies, such as middle cerebral artery occlusion (MCAO) [3,13,18,20,21], common carotid artery occlusion (CCAO) [12,15], bilateral CCAO [7,9], and four vessel occlusion (4-VO) [14,17,19]. MCAO was conducted by inserting a nylon suture into the MCA from internal carotid artery (ICA), and reperfusion was induced by removing the nylon suture at the end of ischemia period. CCAO or bilateral CCAO was induced by obstructing both sides or a single side of common carotid artery. Similarly, in a 4-VO model, rat vertebral arteries of both sides were eletrocoagulated and bilateral common carotid arteries were obstructed by suture; then the sutures were removed for reperfusion (Table 1).

Among the models mentioned above, MCAO has been used most commonly, because the clinical morbidity of MCAO is higher than other vessel occlusions, and it can better simulate the condition of a stroke patient. CCAO and bilateral CCAO are much easier to conduct than MCAO, but they are less clinically relevant because the morbidity of both CCA and bilateral CCA occlusion are less than MCAO [22]. Additionally, 4-VO may cause a total global ischemia and lead to much more severe brain damage, resulting in an instable model. So, the best animal model for the research of hydrogen against cerebral ischemic injury needs to be confirmed through further investigation.

Another model commonly used was a cardiac arrest model [8,10,11,16]. Ventricular fibrillation is induced for several minutes in this model, followed by a return of spontaneous circulation (ROSC). This process will cause total global ischemia of brain with I/R injury. It can also cause ischemic injury of other organs and tissues, so some parameters, such as survival rate of animals and concentration of cytokine, may be influenced. The significance of these parameters will be weakened in measuring the degree of cerebral I/R injury.

2.2.3. Effect of hydrogen on survival rate and behavior of animal

The survival rate after reperfusion can be increased by hydrogen administration in animal studies. According to research by Nagatani et al. (Table, number 9), the 7-day survival rate of mice after bilateral CCAO was 8.3%. Inhalation of 1.3% hydrogen gas significantly improved the survival rate to 50% [7].

Neurofunctional outcomes for the animal was a parameter of prognosis for reperfusion treatment. Ohsawa et al. used neurological score (NS), which was graded from 0 to 5, to measure such outcomes [3]. The highest score, 5, represented death and the lowest score 0 represented no neurological deficits to the mouse. A decrease in the score, neurological outcomes improved. Results showed a significant decrease of NS after the treatment of hydrogen gas inhalation in mice with brain I/R injury [3,16].

Neurological deficit score (NDS) is another parameter of neurological function. It is scored from 0 to 100, with 0 representing the best outcome and 100 the worst. Hayashida et al. (Table, number 13). showed that NDS was significantly increased in control group and both hydrogen treatment and therapeutic hypothermia (TH) decreased the NDS [10]. A better result came from the group of combined application of hydrogen and TH. Neurological Severity Score (NSS) is also used to measure neurological deficit. A score of 13–18 points on NSS represents severe injury; 7–12 moderate injury; and 1–6 mild injury [10]. NSS was used in research by Huang et al. (Table, number 10) to illustrate the therapeutic effect of hydrogen administration. Morris water maze was also used to measure the learning and memory ability of mice. In the research of Cai et al. (Table, number 2), the escape latency of mice in water maze was measured 24 h after hypoxia ischemia (HI). Hydrogen saline treatment shortened the escape latency caused by HI, from 27 ± 3.21 s to 15 ± 1.89 s [15].

2.2.4. Effect of hydrogen on brain tissue morphology

Triphenyl tetrazolium chloride (TTC) staining is a common method used to measure the infarction area in the whole brain. Living tissues are stained red by TTC, but dead tissues are not stained and remain white. Hydrogen treatment reduced the white areas induced by brain injury according to the TTC staining results, which means hydrogen reduced the infarction area after injury [3,13,15,18,20].

Hematoxylin-eosin (HE) staining helps researchers observe the morphological changes in different regions of tissues. Destruction and swelling were observed in brain tissues after I/R injury, such as CA1 area of hippocampus and cortex [9,13,14]. Nissl staining can better show the neuron number in tissues. The number of Nissl-stained positive cells was decreased in tissue with I/R injury, which was ameliorated by hydrogen treatment [13,15,20]. Hayashida et al. used immunohistochemistry staining with anti–neuronal nuclei antibody (NeuN) to mark living neurons. Hydrogen treatment attenuated the decrease of NeuN positive cells, and the effect of combination of hydrogen and TTM was even better [8].

2.3. Mechanism of the protective effect of hydrogen

2.3.1. Anti-oxidative effect of hydrogen treatment

Anti-oxidative effect of hydrogen was demonstrated by Ohsawa et al. [3] in 2007(Graphical Abstract). According to this study, in cultured cells, hydrogen selectively reduced ·OH after the administration of antimycin A. In a cell free system, $ONOO^-$ could also be reduced by hydrogen. Different biomarkers of oxidative stress were measured to illustrate the effect of hydrogen. 8-hydroxy-2 deoxyguanosine (8-OHDG) could be decreased by hydrogen, which indicated that hydrogen could reduce the oxidation of DNA [3,7,13,16,20]. Lipid oxidative biomarkers Hydroxynonenal (HNE) and Malondialdehyde (MDA) were reduced by hydrogen therapy [7,9,14–16,20]. 8-iso-PDF2a [9] and 3-nitrotyrosine (3-NT) [13,20], were also decreased after hydrogen treatment.

Antioxidant enzymes are also used as biomarkers of oxidative stress. In a study by Ge et al. (Table, number 6), the activities of superoxidase (SOD) and catalase (CAT) in the ischemic rats were significantly reduced. Hydrogen attenuated the reduction of SOD and CAT [9,16]. Interestingly, according to research by Hugyecz et al. (Table, number 3), the concentration of manganese superoxide dismutase (MnSOD) was not influenced significantly by transient global cerebral ischemia (TGCI). Expression of MnSOD in the hippocampus was reduced by applied treatments, while its expression in the cortex was only influenced by rosiglitazone (RSG) [12].

Additionally, Nrf2 was shown to participate in anti-oxidation. Zhai et al. demonstrated that oral intake of lactulose could induce hydrogen generation in digestive system [20]. The generated hydrogen reduced oxidative stress, manifested as a reduction of 8-OHdG, MDA and 3-NT. These effects were accompanied with increased expression of Nrf2 mRNA and protein. The administration of antibiotics reduced the generation of hydrogen and abolished the reduction of 8-OHdG, MDA and 3-NT. At the same time, the expression of Nrf2 was decreased with the reduction of hydrogen. In summary, hydrogen could reduce oxidation via the Nrf2 pathway.

2.3.2. Anti-inflammatory effect of hydrogen treatment

Anti-inflammation is an important mechanism of hydrogen therapy accompanied with anti-oxidation (Graphical Abstract). As shown in research by Ohsawa et al., the number of ionized calcium binding adapter molecule 1 (Iba-1) and glial fibrillary acidic protein (GFAP) positive cells were increased after I/R injury, indicating that microglia and astrocytes were proliferated and assembled by inflammatory

reaction [3]. Hydrogen decreased the number of microglia and astrocytes in injured brain tissues [8,15]. In another study by Li et al. (Table, number 16), hydrogen saline treatment increased the number of $CD4^+CD25^+FOXP3^+$ regulatory T cells, which had an anti-inflammatory effect on the blood after brain I/R injury [19]. These results illustrated that hydrogen can regulate proliferation of anti- or pro-inflammatory cells.

Besides cellular activation, hydrogen can also regulate cytokines. After cerebral I/R injury, level of cytokines in serum or tissue, such as tumor necrosis factor-α (TNF-α), interleukine-6 (IL-6) and interleukine-1β (IL-1β), increased due to inflammation [13,14]. These pro-inflammatory cytokines were decreased by hydrogen treatment. However, anti-inflammatory cytokines, such as transforming growth factor-1β (TGF-1β), were also increased by hydrogen treatment [19]. Finally, nuclear factor κB (NF-κB) is one of pro-inflammatory transcription factors. Hydrogen treatment reduced number of NF-κB positive cell, indicating that hydrogen could affect pro-inflammatory gene expression [14,19].

2.3.3. Anti-apoptotic effect of hydrogen treatment

Anti-apoptosis is also a mechanism of hydrogen treatment (Graphical Abstract). TdT-mediated dUTP Nick-End Labeling (TUNEL) is a classic way to measure apoptosis. The number of TUNEL positive cells was shown to be increased in the cortex and hippocampus after hypoxia-ischemia, and it was reduced with hydrogen water treatment [13,16,20]. The immunohistochemistory results staining with caspase-3 were consist with these TUNEL results [13–15,20].

2.3.4. Other effects induced by hydrogen treatment

Hydrogen can influence the expression of miRNA (Graphical Abstract). MiR-210 has been identified as a specific hypoxia-related miRNA, and MiR-21 is an effective anti-apoptotic factor. In research by Li et al., miRNAs were measured as parameters of brain injury. I/R injury was shown to elevate the levels of MiR-210 and MiR-21, which were then reduced by hydrogen treatment [19].

The expression of some proteins could be affected by hydrogen treatment (Fig. 1). Cyclooxygenase-2 is a kind of inflammatory enzyme involved in prostaglandin synthesis. Hugyecz et al. demonstrated that hydrogen inhalation reduced the level of COX-2 after TGCI [12]. Similarly, connexins are proteins consisting of gap junctions in brain. According to previous study, inhibition of gap junction can reduce TGCI-induced injury in rats. Hugyecz et al. measured connexin 30 and 43 in brain of rats and showed a reduction of connexin 30 in hippocampus induced by hydrogen treatment. However this effect was not observed in the cortex. Interestingly, over-expression of connexin 43 was reduced by hydrogen treatment in both hippocampus and cortex [12].

Ultrastructural changes to the mitochondria occurring after hydrogen treatment were reported by Cui et al. (Table, number 12; Fig. 1) [17]. According to their findings, mitochondria became swollen and had an irregular shape after I/R. In isolated mitochondria without Ca^{2+}, no significant decrease of mitochondrial transmission value was observed. After administration of 200 μmol/l Ca^{2+}, their transmission value was significantly decreased. Additionally, hydrogen-rich saline administration attenuated the decrease of mitochondrial transmission value, and this effect was reversed by administrating atractyloside, an activator of mPTP channel. In contrast with the I/R group, the hydrogen-rich saline treatment had increased mitochondrial membrane potential and reserved cytochrome *c* inside mitochondria, and this effect were also reversed by atractyloside. These results indicated that hydrogen-rich saline could protect mitochondria from swelling and dysfunction by attenuating mPTP opening in cerebral I/R injury.

Parvalbumin and hippocalcin are responsible for the maintenance of Ca^{2+} concentration during I/R injury. In the study of Han et al. (Table, number 14), hydrogen-rich water significantly attenuated the reduction of parvalbumin and hippocalcin, and it increased the number of parvalbumin and hippocalcin positive cells after MCAO (Fig. 1) [18].

3. Clinical research about hydrogen

3.1. Effectiveness and safety of using hydrogen

In a study by Ono et al., hydrogen concentration was measured by gas chromatography on three patients before, during and after 4% or 3% hydrogen gas inhalation [23]. In these three patients, hydrogen concentration in the blood reached a plateau level 20 min after

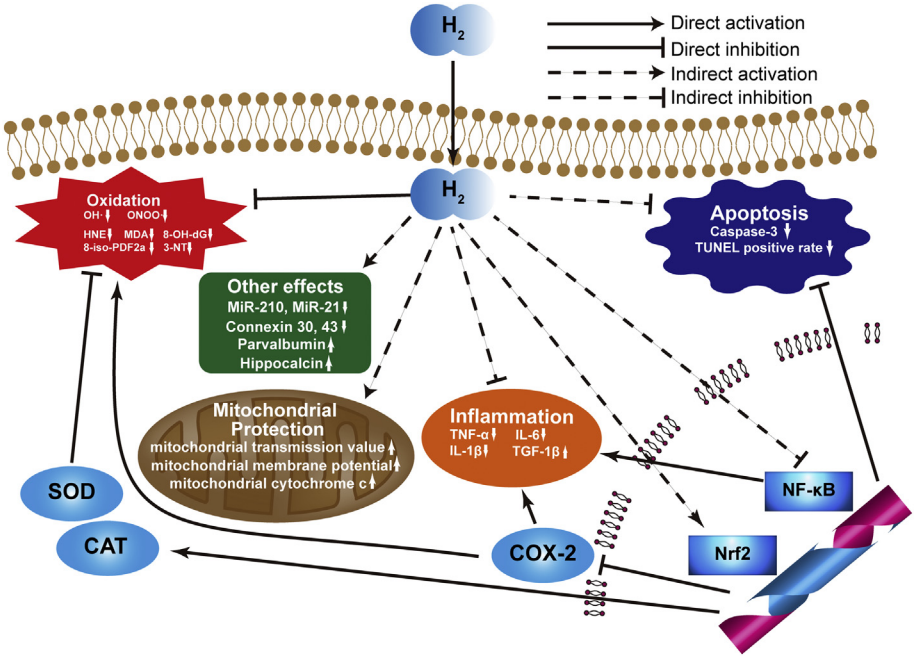

Fig. 1. This figure briefly summarized the mechanism of hydrogen against cerebral I/R injury. As shown in the figure, hydrogen generated anti-oxidative effect directly and other effects indirectly, including anti-inflammation, anti-apoptosis, mitochondrial protection and gene expression regulation.

inhalation, and then rapidly decreased to 10% of the plateau level at 6 and 18 min, in artery and venous blood respectively. Researchers also measured the hydrogen concentration in the venous blood of 10 patients after 30-min inhalation of hydrogen.

An open-label, prospective, non-randomized study of intravenous hydrogen-administration was completed by Nagatani et al. [24]. Their aim was to initially estimate the side-effects and benefits of using hydrogen treatment on patients with ischemic stroke. Thirty-eight patients were involved in this trial where hydrogen-enriched glucose-electrolyte solution with edaravone was administrated to patients immediately after the diagnosis of acute ischemic stroke. Patients with acute stroke within three hours after onset received intravenous t-PA treatment. Hydrogen treatment and edaravone were administrated before t-PA treatment. No significant adverse effects of hydrogen therapy were observed in these patients.

Additionally, MDA-LDL concentration in the blood was slightly reduced by hydrogen treatment. However, patients with infarction of the basal ganglia regions in the anterior circulation, corona radiate, or brain stem and thalamus, showed a significant decrease in MDA-LDL. According to these results, hydrogen treatment was safe for patients with ischemic stroke, regardless of whether they received t-PA treatment or not.

3.2. Initial clinical trials on hydrogen treatment for ischemic stroke

Ono et al. published a randomized controlled trial (RCT) on hydrogen therapy [25]. In their study, they measured the effect of hydrogen on ischemic stroke. Fifty patients were included in this trial who had a NIHSS score between 2 and 6. They were divided into two groups; the hydrogen treatment group received inhalation of 3% hydrogen with ozagrel treatment, and the control group received edaravone with ozagrel or argatroban. The treatment time window was 6 to 24 h after stroke onset. In the first seven days after ischemia onset, patients in treatment group received 3% hydrogen inhalation twice a day, for one hour each time.

There were no side effect were observed among these patients. The relative signal intensity of MRI was lower in hydrogen treatment group in contrast to the control group, and those in the hydrogen treatment group recovered more quickly than the control group. Moreover the treatment group, NIHSS score began to decrease on day 3, earlier than control group, and the functional recovery was more remarkable than in the control group. Activities of daily living (ADL) capability, measured by Barthel Index (BI), Brunnstrom Stage (BRS), modified Rankin Score (mRS), and Functional Independence Measure (FIM), also improved with hydrogen treatment.

4. Discussion

Ischemic stroke has become the second leading cause of disease mortality in the world, after only ischemic cardiac disease [1]. Technologies, such as carotid endarterectomy and carotid artery stent, were first introduced in the last 10 to 20 years, effectively preventing the occurrence of ischemic stroke by minimizing clot obstruction [26,27]. As for acute phase stroke treatment, intravenous treatment of rt-PA and endovascular treatment have been used as reperfusion therapy [28–30]. However, the number of patients who are able to receive endovascular treatment is low, and the time-window for reperfusion treatment is still very narrow [29,30]. Additionally, the benefits of reperfusion treatment are limited due to I/R injury. Neurons near the ischemic core begin necrosis within six minutes after onset of ischemic stroke. In the areas surrounding the ischemic core, ischemic penumbras exist where function is generally intact. However, release of glutamate and free radicals, calcium channel dysfunction, membrane disruption, inflammation, and necrotic or apoptotic cell death do occur in these areas [31]. In this case, reperfusion can lead to a worse outcome after treatment because of oxidative stress, inflammation and apoptosis. Therefore, a complementary therapy to reduce I/R injury needs to be identified. Lots of researches came out recently focusing on I/R injury. Drugs, such as caspase-3 inhibitor [32], PI3K/Akt pathway activator [33], AMPK regulator [34], and free radical scavengers [35], have proved to have therapeutic effect against I/R injury in animal models. However, the effects of these drugs are usually very limited, and the side effects of them have not been illustrated [36,37].

For these reasons, hydrogen treatment is an ideal choice for complementary therapy. Molecular hydrogen is an elementary substance with reducibility. According to previous studies, this molecule can ameliorate cell death induced via I/R injury and morphological changes [38]. Anti-oxidative, anti-inflammatory and anti-apoptotic effects are three major mechanism of hydrogen. They can selectively eliminate free radicals and ameliorate oxidative stress by increasing anti-oxidative enzymes and activating Nrf2 pathway in vitro and in vivo. Pro-inflammatory cells and cytokines can be reduced by hydrogen, and the expression of inflammation-related nuclear factors can also be inhibited. Furthermore, the number of apoptotic cells is reduced by hydrogen treatment. Other mechanisms include reducing the expression of COX-2 as well as connexin 30 and 43, protecting against ultrastructural damage in mitochondria by attenuating met opening, and reversing the reduction of parvalbumin and lipocalin. Importantly, different from other drugs, hydrogen is non-toxic to human. Clinical research proved that inhalation of hydrogen didn't lead to harmful side-effect on human [23,24]. So, hydrogen may be the ideal complementary therapy we are looking for.

However, though many mechanisms of hydrogen have been illustrated, and several clinical trials have been conducted, there remain some issues regarding hydrogen therapy; for example, hydrogen administration. In animal experiments, hydrogen was administrated through inhalation of hydrogen gas, injection of hydrogen-rich water, and oral intake of lactulose. Studies of inhalation therapy included different concentrations of hydrogen, such as 1%, 2%, 1.3%, 4%, and 66.7%. According to the study by Ohsawa et al., 2% hydrogen inhalation presented had the best effect of reducing infarct volume among concentration of 1%, 2% and 4% [3]. However, this did not mean that 2% hydrogen inhalation was the best concentration of hydrogen treatment. The best concentration remains unknown. Another method of administration, injection of hydrogen-rich water or saline, is also used widely in studies. Hydrogen-rich water is produced by dissolving hydrogen into saline with high pressure. However, the pressure used to dissolve hydrogen and ventilation time were various among these researches. There is no uniform standard about production and administration of hydrogen-rich water.

Another issue is that the precise mechanism of hydrogen remains unclear. The anti-oxidative effects of hydrogen have been illustrated to be induced by elimination of free radicals. However in some studies, hydrogen treatment could also increase the level of anti-oxidase. After injury of the brain, abundant free radicals can damage the neuronal function and reduce the activity of anti-oxidases such as CAT and SOD. Therefore, the effect of increasing anti-oxidase may be a direct result of hydrogen's effect on free radicals, and it may also be the indirect result of the anti-oxidase expression. In a study by Zhai et al., lactulose-induced hydrogen activated Nrf2 pathway was proven to increase the level of several anti-oxidase including heme oxygenase (HO-1), SOD and CAT [20]. This may be one of the mechanisms by which hydrogen therapy acts but further investigation is needed to determine how hydrogen activates Nrf2 pathway and which receptor or molecule is its target.

Current clinical researches about hydrogen against ischemic stroke are insufficient. Several trials have shown that hydrogen treatment is not only safe but also effective [24]. Another preliminary clinical trial illustrated that the effect of hydrogen were better than treatment with edaravone, a drug used in stroke recovery [25]. Although this was an RCT, the scale of the trial was not large enough to be conclusive, as it only involved 25 patients in each group.

To solve the problems mentioned above, more efforts should be made. Further investigation is needed to evaluate the hydrogen concentration and its changing curve in blood, in order to define weather a higher concentration of hydrogen inhalation can improve the blood hydrogen concentration or promote an increase in velocity. The production criterion and the qualification standard of hydrogen-rich water also need to be established. The same goes for standardization of inhalation methods and blood hydrogen measurement after injection of hydrogen-rich water. In ischemic stroke, oral intake of hydrogen seems unsuitable for treatment. Thus, inhalation of hydrogen and injection of hydrogen-rich water deserve further investigation.

As for the mechanism of hydrogen, the source of the therapeutic effect of hydrogen needs to be determined. Besides Nrf2 pathway, many molecules associated with the mechanism of hydrogen therapy, such as Lyn, MEK, ERK, p38, JNK, ASK1, Akt, GTP-Rac1, iNOS, Nox1, NF-κB, IκBα, STAT3, NFATc1, c-Fos, GSK-3β and ROCK, have been identified in several animal models [39]. Without exception, these mechanisms are involved in the anti-oxidative, anti-inflammation, and anti-apoptotic effects of hydrogen. Whether these mechanisms are the cause of the anti-oxidative effects of hydrogen or just a consequence remains undetermined, along with the anti-inflammatory, anti-apoptotic effects and changes in many other molecules. Further research should illuminate whether these effects are the direct consequence of anti-oxidation or the consequence of activation of several pathways.

More large scale RCT clinical research is needed before hydrogen therapy can become widespread to treat ischemic stroke. Tamura et al., designed an RCT with a larger scale [40]. (This protocol hasn't been operated.) In his protocol, 360 patients who had suffered from a coma because of cardiac arrest were included in the study. The patients were randomly separated into a control group and hydrogen therapy group. Patients in the control group received high concentrations of oxygen (24–50%) and target thermal management (TTM) treatment, while patients in the hydrogen treatment group received 2% hydrogen inhalation and a high concentration of oxygen. After 90 days of observation, Cerebral Performance Categories scale, survival rate, and other neurological parameters were measured to estimate the effect of hydrogen treatment. More larger scale trials like this need to be performed in the future.

In conclusion, hydrogen treatment is an excellent complementary therapy in the treatment of ischemic stroke. Further study is needed to set the appropriate criterion for use of hydrogen, to illustrate the precise mechanism of hydrogen therapy, and more trials need to be performed studying hydrogen. With these achievements, we can look forward to successful patient outcomes using hydrogen to treat ischemic stroke.

Acknowledgement

This paper was funded by Shanghai Health System Excellent Talents Program (NO. 2017YQ034) and Shanghai Pujiang Program (NO. 16PJD003).

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