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REVIEW

Hydrogen therapy as a potential therapeutic intervention in heart disease: from the past evidence to future application

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Abstract

Cardiovascular disease is the leading cause of mortality worldwide. Excessive oxidative stress and inflammation play an important role in the development and progression of cardiovascular disease. Molecular hydrogen, a small colorless and odorless molecule, is considered harmless in daily life when its concentration is below 4% at room temperature. Owing to the small size of the hydrogen molecule, it can easily penetrate the cell membrane and can be metabolized without residue. Molecular hydrogen can be administered through inhalation, the drinking of hydrogen-rich water, injection with hydrogen-rich-saline, and bathing of an organ in a preservative solution. The utilization of molecular hydrogen has shown many benefits and can be effective for a wide range of purposes, from prevention to the treatment of diseases. It has been demonstrated that molecular hydrogen exerts antioxidant, anti-inflammatory, and antiapoptotic effects, leading to cardioprotective benefits. Nevertheless, the exact intracellular mechanisms of its action are still unclear. In this review, evidence of the potential benefits of hydrogen molecules obtained from in vitro, in vivo, and clinical investigations are comprehensively summarized and discussed with a focus on the cardiovascular aspects. The potential mechanisms involved in the protective effects of molecular hydrogen are also presented. These findings suggest that molecular hydrogen could be used as a novel treatment in various cardiovascular pathologies, including ischemic–reperfusion injury, cardiac injury from radiation, atherosclerosis, chemotherapy-induced cardiotoxicity, and cardiac hypertrophy.

Keywords Molecular hydrogen · Ischemia · Oxidative stress · Inflammation · Cell death · Apoptosis

Introduction

Molecular hydrogen is the lightest of all gas molecules. It is an odorless, colorless, tasteless, nonmetallic, and nontoxic gas at room temperature [1]. Hydrogen is not dangerous when its concentration is under 4% [1]. Owing to its small size, a hydrogen molecule has the ability to diffuse through the cell membrane and enter the cytosol. This characteristic of hydrogen makes it superior when it comes to the transport efficacy of most hydrophilic compounds, which are retained at membranes and cannot reach the cytosol; the majority of hydrophobic ones cannot penetrate biomembranes without specific carriers [2, 3]. Many antioxidants, including vitamins, can enter the cytoplasm but not the mitochondria [2]. It has been shown that hydrogen can be rapidly distributed into the cytosol and organelles, and it can enter the mitochondria and nucleus with excellent efficacy and lack of adverse effects [3].

There is growing evidence to demonstrate that hydrogen could be an effective treatment in various diseases due to its ability to reduce oxidative stress by selectively eliminating

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toxic reactive-oxygen species (ROS) and reactive nitrogen species (RNS) [4]. Hydrogen was found to increase the level of antioxidants in vitro studies, animal models, and clinical studies [5–7]. Furthermore, anti-inflammation and anticell death have also been reported as hydrogen properties [8]. In addition, the previous studies in both animal models and clinical trials demonstrated the potential benefits of hydrogen application in various pathological conditions, including postcardiac arrest syndrome [9] and cardiovascular diseases [10–13].

In this review, we comprehensively summarize the reports regarding the potential role of the therapeutic application of molecular hydrogen in the cardiovascular aspect and describe the potential mechanisms responsible for the benefits of hydrogen. These findings from both preclinical and clinical studies will encourage further investigations to warrant the application of hydrogen as a novel treatment in a clinical setting in the near future.

Effects of hydrogen treatment on cardiomyocytes: reports from in vitro studies

Hypoxia and reoxygenation (H/R) induced oxidative stress and inflammatory reaction is one of the main factors contributing to myocardial cell injury [6, 14, 15]. It has been demonstrated that hydrogen exerts antioxidative stress, antiapoptotic, and anti-inflammatory effects [16]. Following 4 h of hypoxia and 24 h of reoxygenation, a hydrogen-rich medium was shown to increase the survival of H9c2 cells by decreasing inflammatory cytokine release and apoptosis [15]. The protective effect of hydrogen against cell death, inflammatory process, or oxidative stress was shown to be through various pathways, including the PI3K/Akt signaling pathway and the activation of the Nrf2/HO-1 signaling, leading to increased OH-1 levels which is considered a potent antioxidant, and a decrease in 8-OHdG which is regarded as an indicator of oxidative stress (Figs. 1 and 2) [6, 14].

In cardiotrophin-I (CT-I)-induced hypertrophy neonatal rat cardiomyocytes, a hydrogen-rich medium effectively reduced cardiomyocyte hypertrophy via down-regulation of IL-6 and activation of the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling pathway, leading to attenuation of adverse cardiac remodeling and the cell inflammatory response (Fig. 1) [17, 18]. These in vitro reports are comprehensively summarized in Table 1.

Effects of hydrogen treatment on the heart: reports from in vivo studies

Hydrogen treatment has been investigated in various in vivo models of cardiac pathology, including cardiac ischemia – reperfusion injury, myocardial infarction and chronic intermittent hypoxia, radiation, atherosclerosis, sepsis, cardiotoxicity from chemotherapy, and cardiac hypertrophy. The cardioprotective effects of hydrogen interventions are reported and summarized in Tables 2 and 3. The potential mechanisms of action of molecular hydrogen on selective antioxidants, anti-inflammation, and alleviating cell death are demonstrated in Figs. 1 and 2.

Effects of hydrogen treatment on cardiac ischemia – reperfusion injury models

Cardiac ischemia – reperfusion injury (I/R) could negatively affect outcomes in various clinical settings, including post myocardial infarction, cardiac transplantation, or cardiopulmonary bypass. Oxidative stress induced by I/R was found to cause direct cellular injury and apoptosis, leading to impaired cardiac function [15]. The oxidative stress involved in the cell death pathway is a consequence of the presence of reactive oxygen species (ROS), including the hydroxyl radical ($^{\bullet}OH$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), in addition to reactive nitrogen species (RNS), including nitric oxide (NO), and peroxynitrite ($ONOO^-$). Both ROS and RNS are known to trigger the production of inflammatory cytokines and proteins, including IL-1 β, IL-6, and TNF-α, HMGB1, and ICAM-1 [19]. Hydrogen has been demonstrated to potentially protect against I/R injury through the mechanisms of reducing oxidative stress, inflammation, and cell death in various in vivo experimental settings.

In rats with cardiac I/R, hydrogen-rich saline (HRS) injected intraperitoneally was shown to improve cardiac function, reduce infarct size, and alleviate cardiac injury [5, 15, 20]. Studies using either injection into the myocardial tissue around the infarct zone or inhalation in rats showed consistently beneficial results [21–23]. In swine with cardiac I/R, inhalation of 2–4% hydrogen treatment resulted in the reduction of both myocardial infarct size and the incidence of ventricular fibrillation (VF)/ventricular tachycardia (VT) and improved cardiac function [24].

In the cardiopulmonary bypass model (CPB), rats treated with hydrogen-rich water (HRW) via intravenous injection showed an improvement in cardiac function and a reduction in cardiac injury [6]. Within the last few decades, studies using the heart transplant rat model have demonstrated that hydrogen given either orally or by inhalation resulted in reduced infarct size and cardiac injury and enhanced the survival of cardiac grafts [11, 25]. Overall, evidence from these in vivo reports indicated that hydrogen treatment effectively reduced infarct size and myocardial injury, leading to improved cardiac function.

The precise mechanisms involved during hydrogen treatment with regard to improving cardiac function and

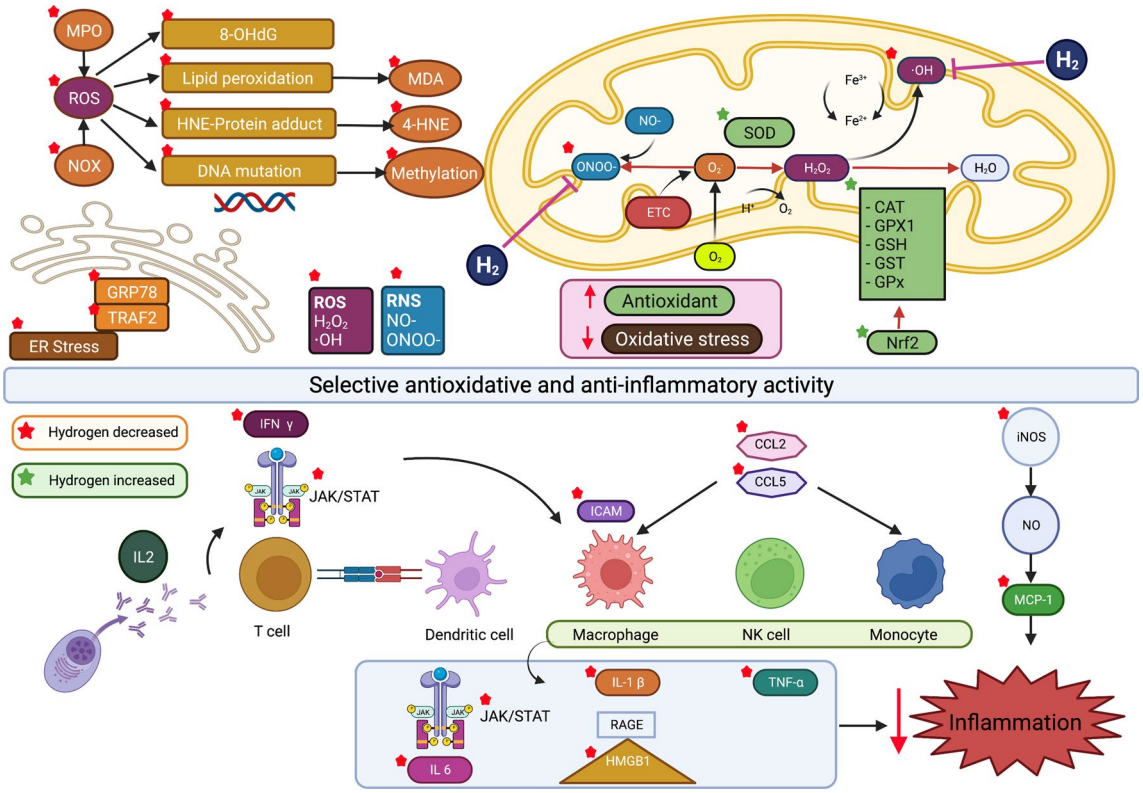

Fig. 1 Potential mechanisms of the action of molecular hydrogen on selective antioxidants and anti-inflammation. The possible mechanisms of molecular hydrogen proposed have been those which increased antioxidants, and decreased oxidative stress, and inflammation. *CAT* catalase, *4-HNE* 4-hydroxyl-2-nonenal, *8-OHdG* 8-hydroxydeoxyguanosine, *CCL* chemokine (C–C motif), *DNA* deoxyribonucleic acid, *ER* endoplasmic reticulum, *ETC* electron transport chain, *GPx* glutathione peroxidase, *GPX1* glutathione peroxidase 1, *GRP78* glucose-regulated protein 78, *GSH* glutathione peroxide, *GST* glutathione-S-epoxide transferase, *HMGB* high mobility group box 1, *ICAM* intercellular adhesion molecule, *IFNγ* interferon γ, *IL* interleukin, *iNOS* inducible nitric oxide synthase, *JAK/STAT* janus kinase/signal transducer and activation of transcription signal pathway, *MCP-1* monocyte chemotactic protein-1, *MDA* malondialdehyde, *MPO* myeloperoxidase, *NK cell* natural killer cell, *NOX* Nox protein, *Nrf2* nuclear factor erythroid 2-related factor2, *·OH* hydroxyl radicals, *RNS* reactive nitrogen species, *ROS* phosphatidylinositol 3-kinase, *SOD* superoxide dismutase, *TNF-α* tumor-necrosis factor-α, *TRAF2* tumor-necrosis factor-a (TNF-a) receptor-associated factor 2

alleviating myocardial infarction and cardiac injury are still unclear, but the reduction in oxidative stress and inflammation could be key [5, 15, 20–22]. Hydrogen has been shown to effectively decrease oxidative stress indicators, including MDA, 8-OHdG, MPO, and ROS in rats with I/R, CBP, and in transplantation models [5, 6, 15, 20–23] as well as decreasing endoplasmic reticulum (ER) stress including TRAF2, and GRP78 in an I/R rat model [5, 6, 15, 20–23]. An increase in antioxidants, including SOD, was also demonstrated in rat models of I/R injury and CBP [5, 6, 15, 20–23]. Hydrogen treatment also led to a reduction in inflammation via increased autophagy, PINK/Parkin-mediated mitophagy [6, 11, 15, 20, 21, 26], and antiapoptosis [5, 6, 11, 15, 21, 23]. All of these mechanisms could lead to improved cardiac function in these models.

Effects of hydrogen treatment in myocardial infarction (MI) and chronic intermittent hypoxia (CIH) models

Myocardial infarction (MI), widely accepted as one of the major causes of death, can induce myocardial necrosis and interstitial fibrosis resulting in heart failure, and increasing the mortality rate [27]. In rats with MI, hydrogen treatment via ingestion, inhalation, or intraperitoneal injection was shown to improve cardiac function and attenuate myocardial pathological changes by reducing the infarct size and apoptosis [28–30]. In rats with CIH, hydrogen therapy has been shown to reduce cardiac dysfunction by reducing oxidative stress. In addition, hydrogen attenuated ER stress-induced apoptosis via PERK-eIF2 α-ATF4, IRE 1-XBP1, and ATF6

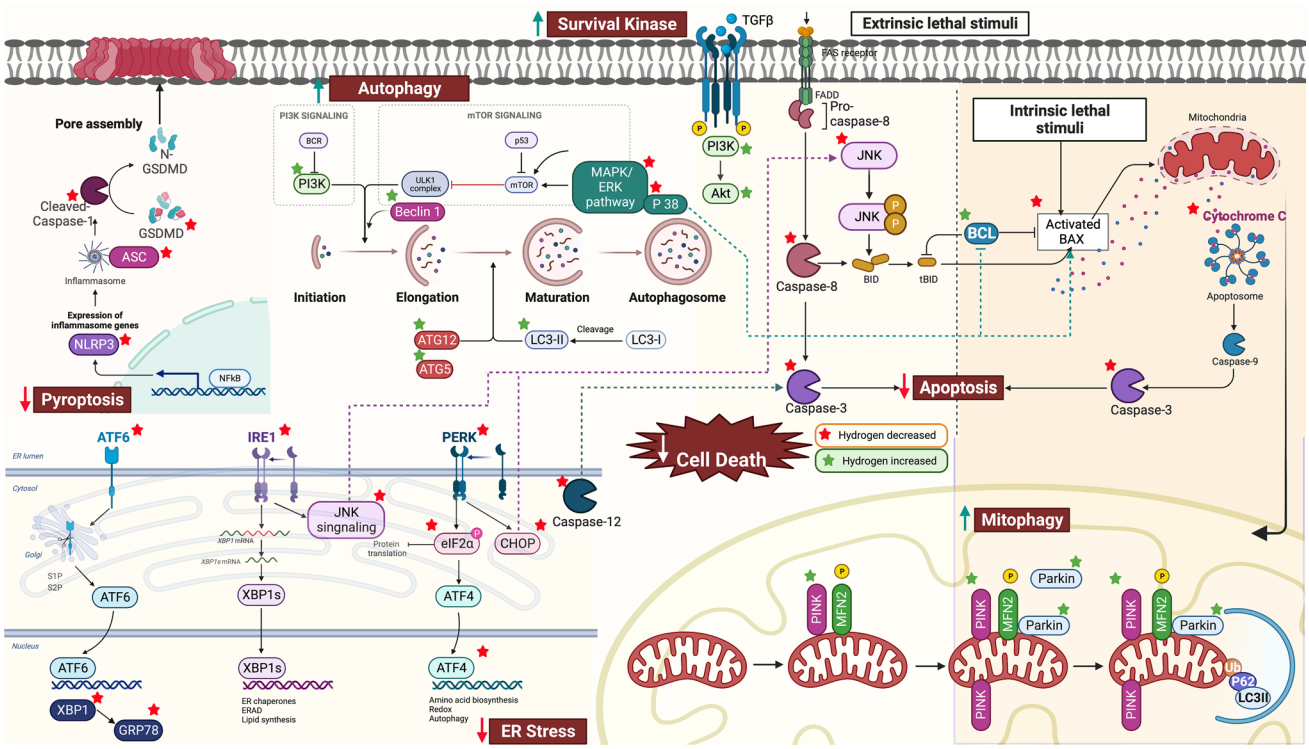

Fig. 2 Potential mechanisms associated with the action of molecular hydrogen in alleviating cell death. It has been proposed that molecular hydrogen effectively decreases apoptosis, ER stress, and pyroptosis. Molecular hydrogen has been shown to increase autophagy, mitophagy, and survival kinases, leading to the alleviation of cell death. *Akt* protein kinase b, *ASC* apoptosis-associated speck-like protein containing a card, *ATF* activating transcription factor, *ATG* autophagy-related protein, *Bax* apoptosis regulator Bax, *Bcl-2* apoptosis regulator Bcl-2, *CHOP* the proapoptotic transcriptional factor c/ebp homologous protein, *elF2 α* eukaryotic initiation factor 2 alpha, *ER* endoplasmic reticulum, *ERAD* endoplasmic-reticulum-associated protein degradation, *ERK* extracellular signal-regulated kinase, *FADD* fas associated via death domain, *GRP78* glucose-regulated protein 78, *GSDMD* gasdermin D, *IRE1* Er stress sensor and cell fate executor, *JNK* c-jun N-terminal kinase, *LC3-I* microtubule-associated protein 1 light chain 3α, *MAPK* mitogen-activated protein kinase, *MFN2* mitofusin-2, *mTOR* mammalian target of rapamycin, *NFkB* nuclear factor kappa-light-chain-enhancer of activated b cells, *NLRP3* nod-like receptor (NLR) family pyrin domain containing protein 3, *P38* 38-kda protein, *P53* 53-kda protein, *P62* 62-kda protein, *PERK* protein kinase RNA-like endoplasmic reticulum kinase, *PI3K* phosphatidylinositol 3-kinase, *PINK* PTEN-induced kinase, *TGF β* transforming growth factor beta, *XBP1* x-box binding protein 1, *XBP1s* active/spliced form of XBP1

pathways [31]. Moreover, a combination of HRS with exercise was shown to promote the repair of both the mitochondria and DNA in a rat MI model, which could be involved in the cardioprotective mechanism of hydrogen treatment [29].

Effects of hydrogen treatment in a radiation model

Radiation can cause myocardial damage as a consequence of radiation-induced myocardial fibrosis, leading to the chronic impairment of cardiac function [32]. In a radiated rat model, it has been demonstrated that an intake of oral hydrogen prior to radiation increased survival rate by increasing the level of antioxidants, reducing oxidative stress, and preventing DNA damage [33]. However, the effect of hydrogen treatment on cardiac function in these conditions is unknown.

Effects of hydrogen treatment in an atherosclerosis model

Atherosclerosis is a multifactorial process which is related to cardiovascular disease. It represents a state of inflammation and oxidative stress characterized by the accumulation of macrophages and oxidized products of lipoproteins in the affected blood vessels [34]. Interestingly, the consumption of HRS for 6 months effectively decreased oxidative stress

Table 1 Effects of hydrogen treatment on cardiomyocytes: reports from in vitro studies

Cell type/Study model	Intervention/Dose/Duration	Major findings					Interpretation	References
		Cell viability	Oxidative stress	Inflammation	Apoptosis	Autophagy/Mitophagy		
H9c2 cells/ H/R model (4 h/24 h)	H_2 gas-rich medium/0.6 mmol/l/ throughout H/R period	↑	–	↓ IL-1β ↓ IL-6 ↓ TNF-α ↓ HMGB1	↓ Caspase 3 ↑ Bcl-2/ Bax ratio	↑ LC3II/I ↑ ATG5 ↑ ATG12 ↑ Beclin 1 ↑ PINK1 ↑ Parkin	H_2 increased cell survival via reduced inflammation, apoptosis, and promoted autophagy and mitophagy in H/R model	[15]
	– Treated with rapamycin (autophagy induction)/20 µM/2 h prior to experimentation and throughout H/R period	↑	–	↓ IL-1β ↓ IL-6 ↓ TNF-α ↓ HMGB1	↓ Caspase 3 ↑ Bcl-2/ Bax ratio			
	– Treated with 3-MA (autophagy inhibitor)/1 mM/2 h prior to experiment and throughout H/R period	↓	–	↑ IL-1β ↑ IL-6 ↑ TNF-α ↑ HMGB1	↑ Caspase 3 ↓ Bcl-2/ Bax ratio			
	– Treated with PINK1siRNA (inhibition of mitophagy)/50 nM/ throughout H/R period	–	–	↑ IL-1β ↑ IL-6 ↑ TNF-α ↑ HMGB1	↑ Caspase 3 ↓ Bcl-2/ Bax ratio			
H9c2 cells/ H/R model (2 h/4 h)	Hydrogen-rich water/0.8 mM/l/3 days prior to H/R, prior to hypoxia and during reoxygenation	↑	↑ HO1	–	↓ BAX ↓ Caspase 3 ↑ Bcl-2 ↑ PI3K ↑ p-AKT	–	H2 increased cell survival and antioxidant levels, and decreased apoptosis through PI3K/Akt signaling pathway in H/R model	[6]
	– Treated with LY294002 (PI3K inhibitor/40 µM/l/throughout H/R period	↓	↓ HO1	–	↑ BAX ↑ Caspase 3 ↓ Bcl-2 ↓ PI3K ↓ p-AKT	–		

Table 1 (continued)

Cell type/Study model	Intervention/Dose/Duration	Major findings					Interpretation	References
		Cell viability	Oxidative stress	Inflammation	Apoptosis	Autophagy/Mitophagy		
H9c2 cells/ Hypoxia model :$CoCl_2$ model (400–800 µM) (24 h)	H_2 gas-rich medium/NA/24 h H_2 gas-rich medium/NA/6, 12, 18 h	↔	–	–	–	–	H_2 did not affect cell viability in $CoCl_2$-induced hypoxia; however, it effectively increased cell viability in SGD-induced ischemia via reduced oxidative stress and promoted antioxidants in an Nrf2 and HO1 dependent manner	[14]
Ischemia model SGD model (6, 12, 18 h)		↑at 6 and 12 h	–	–	–	–		
SGD model (6 h)	H_2 gas-rich medium/NA/30 h	↑	↓ 8-OHdG ↑ HO1 ↑ Nrf2	–	–	–		
	– With ZnPP IX (HO-1 inhibitor)/ 10 µM/30 h	↓	–	–	–	–		
	– With BR (Nrf2 inhibitor)/10 µM/30 h	↓	–	–	–	–		
	– With Si-Nrf2/20 mmol/l/30 h	↓	↓ HO1	–	–	–		
Rat cardiomyocytes CT-1-induced cardiomyocyte hypertrophy	Hydrogen-rich saline/0.6 mmol/l/72 h	–	–	↓ IL-6 ↓ JAK ↓ STAT3	–	–	Hydrogen-rich saline reduced cardiomyocyte hypertrophy and inflammation via the JAK/STAT3 pathway	[17]
	– With AG490 (JAK specific antagonists)/0.1 mM/72 h	–	–	↑ IL-6 ↑ JAK ↑ STAT3	–	–		

Akt protein kinase B, *ATG* autophagy-related protein, *Bax* apoptosis regulator Bax, *Bcl-2* apoptosis regulator Bcl-2, *CoCl2* cobalt chloride, *CT-1* cardiotrophin-1, *HO1* heme oxygenase 1, *HMGB1* high mobility group box 1, *H/R* hypoxia reperfusion model, *HRS* hydrogen-rich saline, *IL* interleukin, *JAK/STAT* Janus kinase/signal transducer and activation of transcription signal pathway, *LC3* microtubule-associated protein 1 light chain 3 *α*, *Nrf2* the nuclear factor erythroid 2-related factor2, *p* phosphorylation, *PI3K* phosphatidylinositol 3-kinase, *PINK* PTEN-induced kinase 1, *SGD* serum and glucose deprivation, *TNF-α* tumor-necrosis factor-*α*, *ZnPPIX* zinc protoporphyrin IX, *8-OHdG* 8-hydroxydeoxyguanosine

Table 2 Effects of hydrogen treatment on cardiac ischemia – reperfusion injury models: reports from in vivo studies

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/ Cardiac injury marker	Oxidative stress	Inflammation	Apoptosis	Autophagy/ Mitophagy		
Male Wistar rats I/R model 30 min/24 h	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 6 h (0.6 mmol/l)/10 ml/kg/5 min prior to reperfusion	IP	↑ HR ↑ MAP ↑ SBP ↑ DBP ↑ LV + dP/dt ↑ LV − dP/dt ↑ LVEF ↓ LVEDP ↓ Infarct size ↓ CK-MB ↓ cTnI	-	↓ IL-1 β ↓ IL-6 ↓ TNF-α ↓ HMGB1	↓ TUNEL ↓ BAX ↓ Caspase 3 ↑ Bcl-2/Bax ratio	↑ LC3II/I ↑ ATG5 ↑ ATG12 ↑ Beclin 1 ↑ PINK1 ↑ Parkin	Hydrogen-rich saline alleviated myocardial infarct size, reduced inflammation, apoptosis, and promoted autophagy and mitophagy, leading to improved left ventricular function and hemodynamics following cardiac I/R injury	[15]
Male SD rats I/R model 30 min/24 h	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 6 h (0.6 mmol/l)/5 ml/kg/5 min before reperfusion	IP	↑ LVSP ↓ LVDP ↑ LV + dP/dt ↑ LV− dP/dt ↓ Infarct size	↓ MDA in tissue and plasma ↓ 8-OHdG	–	↓ TUNEL ↓ Caspase 3	–	Hydrogen-rich saline improved cardiac function and reduced infarct size from I/R injury by reducing oxidative stress and apoptosis	[5]
Male SD rats I/R model 30 min/24 h	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 6 h (0.6 mmol/l)/10 ml/kg/5 min prior to reperfusion	IP	↑ LVSP ↓ LVEDP ↑ LV + dP/dt ↓ LV − dP/dt ↓ Infarct size ↓ PMN accumulation ↓ CK-MB ↓ cTnI	↓ MPO ↓ 3-nitrotyrosine	↓ IL-1 β ↓ TNF-α ↓ ICAM-1	–	–	Hydrogen-rich saline improved cardiac function and reduced infarct size by reducing oxidative stress and inflammation	[20]
Male SD rats I/R model 45 min/3 min, 30 min, or 24 h	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 4 h (60 µL)/NA/at onset of reperfusion	Injected into the myocardial tissue around the infarct zone	↑ LV + dP/dt ↑ LV − dP/dt ↔ Infarct size ↓ CK ↓ CK-MB	↓ MDA ↑ SOD	↓ TNF-α	↓ TUNEL ↓ Cyt-c ↓ Caspase-8 ↓ p-p38 ↓ p-JNK ↓ p-ERK	–	Hydrogen-rich saline improved cardiac function from I/R injury by reducing oxidative stress, inflammation, apoptosis, and regulating the MAPK signal pathway	[21]

Table 2 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/ Cardiac injury marker	Oxidative stress	Inflammation	Apoptosis	Autophagy/ Mitophagy		
Male Wistar rats I/R model 30 min/ 24 h	2% H_2/at onset of ischemia and continue for 60 min after reperfusion	Inhalation	↓ LVEDP ↓ LVEDd ↓ LVESd ↑ IVS ↑ PW ↑ FS ↑ EF ↓ Infract size	↓ 8-OHdG	–	–	–	Inhalation of H_2 improved cardiac function and reduced infarction by reducing oxidative stress after I/R injury	[22]
Male Wistar rats I/R model 1 h/2 h	2% H_2/5 min before reperfusion until 2 h after reperfusion	Inhalation	↓ Infarct size ↓ TnI	↓ 8-OHdG ↓ MDA ↓ ROS ↓ TRAF2 ↓ GRP78	–	↓ p-Bcl-2/ Bcl2	↓ LC3II/I ↓ Beclin 1	Inhalation of 2% H_2 gas attenuated myocardial injury by attenuating ER stress, oxidative stress, apoptosis and autophagy	[23]
	Postischemic conditioning treatment (Four cycles of 1 min reperfusion/1 min ischemia (total time, 8 min) was given at the end of 1 h coronary occlusion)		↓ Infarct size ↓ TnI	↓ 8-OHdG ↓ MDA ↓ ROS ↓ TRAF2 ↓ GRP78	–	↓ p-Bcl-2/ Bcl2	↓ LC3II/I ↓ Beclin 1		
	2% H_2 combined with postischemic conditioning treatment		↓ Infarct size ↓ TnI	↓ 8-OHdG ↓ MDA ↓ ROS ↓ TRAF2 ↓ GRP78	–	↓ p-Bcl-2/ Bcl2	↓ LC3II/I ↓ Beclin 1		
Swine I/R model Myocardial stunning 12 min/90 min	2% H_2/during and after ischemia	Inhalation	↓ Incidence of VF, VT ↑ SS	–	–	–	–	Inhalation of 2% H_2 gas during I/R improved cardiac function and reduced VF/VT incidence from myocardial stunning, while inhalation of 4% H_2 gas during I/R reduced infarct size	[24]
Myocardial infarction 40 min/120 min	4%H_2/during and after ischemia	Inhalation	↓ Infarct size	–	–	–	–		
Male SD rats CBP model (1 h)	Hydrogen-rich water under 0.8 MPa dissolved in saline for 24 h/6 ml/kg/prior to hypoxia and during reoxygenation	IV injection via tail vein	↑ MAP ↑ LV + dP/dt max ↓ LDH ↓ CK-MB	↓ MDA ↓ MPO ↑ SOD	↓ IL-1 β ↓ IL-6 ↓ TNFα	↓ TUNEL ↓ BAX ↓ caspase 3 ↑ Bcl-2	-	Hydrogen-rich water improved cardiac function and reduced cardiac injury by reducing oxidative stress, inflammation, and apoptosis	[6]

Table 2 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/ Cardiac injury marker	Oxidative stress	Inflammation	Apoptosis	Autophagy/ Mitophagy		
Male Lewis rats Heterotopic heart transplantation (I/R model) 6 or 18 h/6 h	1%, 2%, 3%H_2/1 h before ischemia and 1 h after reperfusion 1% H_2 2% H_2 3% H_2	Inhalation	↔ CPK ↓ CPK ↓ CPK	–	–	–	–	The combination of hydrogen and CO therapy reduced infarct size, cardiac injury, and enhanced cardiac graft survival by decreasing oxidative stress, inflammation, and apoptosis	[11]
	CO (After 6 h cold ischemia) – CO: 50 ppm – CO: 250 ppm	Inhalation	↔ CPK ↓ CPK	–	–	–	–		
	Inhaled gas (After 18 h cold ischemia) Mixed H_2 and CO		↓ Infarct size ↓ Macrophage ↓ CPK ↓ cTnI ↑ Transplantation score (3 h after storage) ↑ Graft survival after 7 days	3 h after perfusion ↓ MDA ↓ MPO 6 h after perfusion ↓ MPO	3 h after perfusion ↓ IL-1 β ↓ IL-6 ↓ TNF α ↓ iNOS ↓ HMGB1	↓TUNEL ↓ ED1 ↓ cleaved caspase 3	–		
	H_2 alone	Inhalation	↑ Transplantation score (3 h after storage) ↑ Graft survival after 7 days	3 h after perfusion ↓ MDA 6 h after perfusion ↔ MPO	3 h after perfusion ↔ IL-1 β ↔ IL-6 ↔ TNF α ↔ iNOS ↓ HMGB1	↔ TUNEL ↔ ED1 ↔ cleaved caspase 3	–		

Table 2 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/ Cardiac injury marker	Oxidative stress	Inflammation	Apoptosis	Autophagy/ Mitophagy		
Inbred male LEW (RT1l) and BN (RT1n) rats Heterotopic heart transplantation (I/R model)	Hydrogen-rich water/dose: NA/60 d, 100d	Oral	↑ Viability of cardiac allografts ↑ Tissue ATP ↑ Mito activity ↓ CD3 + T cells ↓ CD68 + macrophages	50 d after transplant ↓ MPO ↓ MDA	↓ IFN γ ↓ TNF α ↓ CCL2 ↓ CCL5 ↓ MCP-1	–	–	Hydrogen-rich water enhanced cardiac allograft survival by reducing intimal hyperplasia, inhibition of T cell proliferation, reduction of oxidative stress and increased tissue ATP and mitochondrial activity	[25]
Male LEW (RT1l) and BN (RT1n) rats Orthotopic Aortic transplantation			↓ Intimal hyperplasia in aortic graft						

AAR area at risk, *Akt* protein kinase B, *ATG* autophagy-related protein, *ATP* adenosine triphosphate, *BAX* apoptosis regulator Bax, *Bcl-2* apoptosis regulator Bcl-2, *BNP* brain natriuretic peptide, *CBF* coronary blood flow, *CCL* chemokine (C–C motif) ligand, *CD* cluster of differentiation, *CK-MB* creatinine kinase-MB, *CO* carbon monoxide, *CPB* cardiopulmonary bypass, *CPK* creatine phosphokinase, *cTnI* cardiac troponin-I, *Cyt-c* cytochrome c, *DBP* diastolic blood pressure, *EF* ejection fraction, *ER* endoplasmic reticulum, *ERK* extracellular signal-regulated kinase, *FS* fractional shortening, *GRP78* glucose-regulated protein 78, *HO1* heme oxygenase 1, *HMGB1* high mobility group box 1, *HRS* hydrogen-rich saline, *HR* heart rate, *ICAM-1* intercellular adhesion molecule 1, *IFN γ* interferon γ, *IL* interleukin, *iNOS* inducible nitric oxide synthase, *IP* intraperitoneal, *IVS* interventricular septum, *JNK* c-Jun-N-terminal Kinase, *LC3* microtubule-associated protein 1 light chain 3 α, *LDH* lactate dehydrogenase, *LV* left ventricle, *LVAWd* LV anterior wall thickness at end-diastole, *LVEDd* LV endodiastolic diameter, *LVEDP* left ventricular end-diastolic pressure, *LV dp/dt* rate of pressure change in left ventricle, *LVSP* LV systolic pressure, *LVDP* LV diastolic/developed pressure, *LVDP* LV developed pressure (LVSP-LVDP), *LVEDd* LV endodiastolic diameter, *LVESd* LV endosystolic diameter, *LVPWd* LV posterior wall thickness in diastole, *LVW* left ventricular weight, *IVS* intraventricular septum diameter, *MAP* mean arterial pressure, *MAPK* mitogen-activated protein kinase, *MCP-1* monocyte chemotactic protein-1, *MDA* malondialdehyde, *Mfn2* mitofusin-2, *MPO* myeloperoxidase, *Nrf2* the nuclear factor erythroid 2-related factor2, *·OH* hydroxyl radicals, *p* phosphorylation, *PERK* protein kinase-RNA-like endoplasmic reticulum kinase, *PI3K* phosphatidylinositol 3-kinase, *PINK* PTEN-induced kinase 1, *PMN* polymorphonuclear neutrophil, *PW* posterior wall thickness, *p38* 38-kDa protein, *ROS* reactive oxygen species, *SBP* systolic pressure, *SD* Sprague Dawley rat, *SOD* superoxide dismutase, *SpO2* pulse oximetry, *SS* segment shortening, *TnI* troponin I, *TNF-α* tumor-necrosis factor-α, *TRAF2* tumor-necrosis factor-a (TNF-a) receptor-associated factor 2, *VF* ventricular fibrillation, *VT* ventricular tachycardia, *8-OHdG* 8-hydroxydeoxyguanosine

and had the potential to decrease atherosclerotic lesions in the aorta [35].

Effects of hydrogen treatment on the heart in a sepsis model

Sepsis is systemic inflammation in response to an infection associated with the cardiovascular system. Cardiac myocytes are involved due to the oxygen consumption of the cell being compromised. Correspondingly, mitochondrial dysfunction occurs, leading to cellular energy depletion [36]. A recent study showed that hydrogen gas treatment reduced mitochondrial dysfunction by up-regulating the protein expression of mitofusin-2 (Mfn2), peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), and protein heme-oxygenase-1 (HO-1) [37]. However, the effect of hydrogen treatment on cardiac function in these conditions is unknown.

Effects of hydrogen treatment in a chemotherapy-induced cardiotoxicity model

Doxorubicin is an anthracycline anticancer drug that can cause cardiotoxicity, a condition known as doxorubicin-induced cardiomyopathy, via oxidative stress, apoptosis, and intracellular calcium dysregulation [38]. The use of HRS via intraperitoneal injection in rats treated with doxorubicin has been shown to improve survival rate and reduce cardiac dysfunction by attenuating oxidative stress, inflammation, and apoptosis [12].

Effects of hydrogen treatment in a cardiac hypertrophy model

Cardiac hypertrophy, consisting of interstitial and perivascular fibrosis, can lead to heart failure, which results in increased mortality [39]. Hypertension is the major factor associated with left ventricular hypertrophy [40]. Studies into cardiac hypertrophy in rat models reported that hydrogen therapy using HRS via IP resulted in a reduction in heart and atrial weight [13, 17, 18, 41]. Hydrogen also decreased the incidence of atrial fibrillation (AF), atrial fibrosis, apoptosis, and inflammation through the downregulation of the JAK-STAT signaling [17, 18]. In another rat model with cardiac hypertrophy, the benefit of hydrogen therapy was shown via a reduction in oxidative stress, the inflammatory process, and angiotensin II, and the preservation of mitochondrial function in the left ventricle [13, 41]. These benefits could be due to the inhibition of the TGF-β/Smad signaling pathway, leading to reduced cardiac hypertrophy [41].

Effects of hydrogen treatment on the heart: reports from ex vivo studies

Heart transplant is one of the causes of I/R injury. A period of cold ischemia due to tissue matching and transportation is inevitable after retrieval of the heart. The organ preservation solutions have been found to only partially alleviate ischemia injury during storage [42]. In isolated hearts mounted on the Langendorff apparatus for aerobic perfusion, it has been shown that preservation in H2-rich with Histidine – Tryptophan – Ketoglutarate (HTK) significantly improved cardiac function in a hydrogen concentration-dependent manner as well as attenuated the microscopic pathology of the myocardium [43]. The protective mechanism of hydrogen was via inhibition of cold ischemia-induced up-regulation of oxidative stress, inflammation mediators, and apoptosis (Figs. 1 and 2) [43]. In a study using syngeneic heart grafts from elderly donors or allografts from adult donors and exposing them to prolonged cold preservation, the cardiac grafts immersed in the cold-water bath with hydrogen showed ameliorated myocardial injury [26]. The grafts exhibited inflammatory responses, including neutrophil infiltration, and increases in pro-inflammatory cytokines and chemokines, whereas hydrogen induced lower levels of mitochondrial damage and higher adenosine triphosphate content [26]. In a recent study using an isolated heart model with I/R injury, perfusion with HRW resulted in a decrease in apoptosis by up-regulating the JAK-STAT and PI3K-AKT signaling pathways (Fig. 2) [44]. All of these ex vivo reports are comprehensively summarized in Table 4.

Effects of hydrogen treatment on the heart: Evidence from clinical studies

Because molecular hydrogen has various potential therapeutic effects, it has been investigated in various pathophysiological conditions in clinical settings. It has been suggested that hydrogen has an effective therapeutic approach in the heart for improving outcomes associated with I/R injury. A randomized single-center prospective, open-label, blinded study to investigate the feasibility and effects of hydrogen on the infarct size and adverse left ventricular (LV) remodeling in patients with ST-elevated MI (STEMI) was conducted after primary percutaneous coronary intervention (PCI) [10]. This first clinical trial showed that hydrogen inhalation during PCI is genuinely feasible, promotes LV reverse remodeling 6 months after STEMI, and improves cardiac function [10]. Another recent clinical trial enrolled

Table 3 Effects of hydrogen treatment on the heart: Reports from in vivo studies 'other models"

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/Cardiac injury marker	Oxidative stress	Inflammation	Cell death	Others		
Male Wistar rats MI model Isoproterenol (Iso) twice/subcutaneously at interval 24 h/200 mg/kg	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 6 h/5,7.5, 10 ml/kg/ before Iso administered	IP	↑ LVSP ↓ LVEDP ↑ LV + dP/dt max ↑ LV − dP/dt max ↓ Infarct size ↓ CK-MB ↓ AST	↓ MDA ↓ 8-OHdG ↑ SOD	↓ IL-6 ↓ TNF-α	–	↑ Na + -K + -ATPase activity ↓ Ca2 + -ATPase activity	Hydrogen-rich saline exerted cardioprotective effects against isoproterenol-induced MI by reducing oxidative stress and inflammation	[28]
Male SD rats MI model Ligated LAD	Hydrogen-rich saline under 0.8 MPa concentration of H_2 1.6 ppm/10 ml/kg/daily for 3 wk and additional 30 min before running training	Oral	↑ LVSP ↓ LVEDP ↑ LV + dP/dt ↑ LV − dP/dt ↑ HC ↓ Infarct size ↓ CK-MB ↓ cTnI ↓ h-FABP Myocardial ultrastructural lesion – Normal structure – Sarcomere and Z line regularly arranged and had no outspread phenomenon	↓ MDA ↓ CAT ↑ SOD ↑ GSH ↑ T-AOC	–	–	↑ mt DNA repairase OGG1 ↔ TOM 40 ↑ TOM 20 ↑ TIM 23	A combination of hydrogen-rich saline with exercise ameliorated cardiac dysfunction and injury by reducing oxidative stress and promoting mitochondrial DNA repair	[29]
Male Wistar rats MI model Ligated LAD	2% H_2/24 h after the ligation	Inhalation	↓ LVDd ↓ LVDs ↑ EF ↑ FS ↓ Infarct size ↓ BNP ↓ TnI	↓ MDA ↓ 8-OHdG ↓ ROS	↓ IL-1 β ↓ inflammation cell	↓ TUNEL ↓ NLRP3 ↓ Cleaved-Caspase-1 ↓ ASC ↓ GSDMD	-	Inhalation of 2% H_2 gas alleviated myocardial infarct size and promoted heart function in AMI rats by attenuating inflammation, oxidative stress and pyroptosis	[30]

Table 3 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/Cardiac injury marker	Oxidative stress	Inflammation	Cell death	Others		
Male SD rat CIH model 20 times/h for 8 h/day	H_2-O_2 mixture (67% hydrogen and 33% oxygen)/2 h/day for 35 d	Inhalation	↑ EF ↓ LVEDd ↓ Collagen volume fraction in LV	↓ MDA ↓ NOX2 ↑ SOD ↑ GSH	–	↓ Apoptotic cell ↑ Bcl2/BAX ↓ Caspase 3 ↓ p-JNK ↓ CHOP ↓ GRP78 ↓ Caspase 12 ↓ p-PERK ↓ p-eIF2α ↓ p-IRE 1 ↓ ATF 4 ↓ ATF 6 ↓ XBP 1	–	Treatment with an H_2-O_2 mixture reduced cardiac dysfunction by reducing oxidative stress, ER stress and apoptosis	[31]
Male BALB/c mice Radiation model Radiated with ^{60}Co-gamma rays with a dose rate of 7 Gy	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 6 h/NA/24 h before radiation	Oral	–	–	–	–	↑ Survival rate	Hydrogen-rich saline increased survival rate and alleviated myocardial degeneration caused by radiation-induced myocardial injury through a reduction of oxidative stress and DNA damage	[33]
Radiated with ^{60}Co-gamma rays with a single dose of 15 Gy locally to the heart	Assessment at 100 d		↓ Myocardial degeneration	–	–	–	–		
Radiated with ^{60}Co-gamma rays with a single dose of 6 Gy	Assessment at 4 h		–	↑ SOD ↑ GSH	–	–	–		
	Assessment at 24 h		–	↓ 8-OHdG ↓ MDA	–	–	↓ DNA damage		
Apolipoprotein E-deficient (apoE$^{-/-}$) mice	Hydrogen-rich saline under 0.4 MPa/4.3 ml/day/6 mo	Oral	–	↓ 4-HNE	–	–	↓ Atherosclerotic lesion in the aorta	Hydrogen-rich saline decreased oxidative stress and prevented the formation of atherosclerosis	[35]

Table 3 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/Cardiac injury marker	Oxidative stress	Inflammation	Cell death	Others		
Male Wild type (WT) Sepsis model Cecal ligation and puncture (CLP)	2% H2/ 60 min at the 1 h and 6 h time points after the procedure	Inhalation	–	↑ HO1	–	–	↑ RCR ↑ ATP ↑ MMP ↑ Mfn2 ↑ PGC-1 α ↓ Drp1	Inhaled H_2 attenuated mitochondrial dysfunction associated with severe sepsis by promoting antioxidants through increased HO-1 and Nrf2	[37]
Male Nrf2 knockout (KO) mouse model/CLP				↓ HO1			↓ RCR ↓ ATP ↓ MMP ↓ Mfn2 ↓ PGC-1 α ↑ Drp1		
Zinc protoporphyrin IX (ZnPPIX) prior to cecal ligation and puncture (CLP)				↑ HO1			↑ RCR ↑ ATP ↑ MMP ↑ Mfn2 ↑ PGC-1 α ↓ Drp1		
Male Wistar rat Doxorubicin model (IP 2 mg/kg, every 3 days for 30 days)	Hydrogen-rich saline 4 atm for 1 h (0.55 mmol/l)/10 ml/kg/30 d	IP	↓ LVDs ↑ EF ↑ FS ↓ BNP	↓ MDA ↓ ROS	↓ IL-6 ↓ IL-1 β ↓ TNF-α	↓ TUNEL ↓ Bax/Bcl2 ↓ Cleaved caspase 3 ↓ Cleaved caspase 8	↑ Survival	Hydrogen-rich saline improved survival rate and reduced cardiac dysfunction against chemotoxicity by reducing oxidative stress, inflammation, and apoptosis	[12]

Table 3 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/Cardiac injury marker	Oxidative stress	Inflammation	Cell death	Others		
Male SD rat Cardiac hypertrophy model Abdominal aortic constriction (AAC)	Hydrogen-rich saline (0.6 mmol/l)/Low dose:3 ml/kg/6 wk	IP	↓ HW/BW ↓ AW/BW ↓ LVW/BW ↑ AW/HW ↓ Atrial fibrosis ↓ CVF ↓ LVAWd ↓ LVPWd ↑ FS ↓ AF incidence ↓ AF duration	–	↔ IL-6 ↓ JAK ↓ STAT3	–	–	Hydrogen-rich saline reduced pressure overload-induced cardiac hypertrophy in Rats via suppression of inflammation and the JAK/STAT3 pathway, leading to reduced cardiac dysfunction and remodeling	[17]
	High dose:6 ml/kg/6 wk	IP	↓ HW/BW ↓ AW/BW ↓ LVW/BW ↑ AW/HW ↓ Atrial fibrosis ↓ CVF ↓ HR ↓ LVAWd ↓ LVPWd ↑ FS ↓ AF incidence ↓ AF duration	–	↓ IL-6 ↓ JAK ↓ STAT3	–	–		
Male SD rat Cardiac hypertrophy model Abdominal aortic constriction (AAC)	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 6 h (0.6 mmol/l)/3 or 6 mg/kg/6 wk	IP	↓ HW/BW ↓ LVW/BW ↓ fibrosis ↓ CVF ↓ BNP ↓ ANP	–	↓ IL-6 ↓ JAK ↓ STAT3	↓ TUNEL staining cells	–	Hydrogen-rich saline reduced pressure overload-induced cardiac hypertrophy in rats by decreasing apoptosis and suppressing inflammation via the JAK-STAT signaling pathway	[18]

Table 3 (continued)

Study model	Intervention		Major findings					Interpretation	References
	Dose/Duration	Route	Cardiac function/Cardiac injury marker	Oxidative stress	Inflammation	Cell death	Others		
Wistar-Kyoto rat Cardiac hypertrophy model Spontaneously hypertensive rats (SHR)	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 4 h (0.6 mmol/l)/6 ml/kg/3 mo	IP	↓ LVW/BW	↓ serum MDA ↓ LV MDA ↓ LV ROS ↓ LV OONO- ↑ SOD ↑ GPx ↑ GST ↑ CAT	↓ IL-6 ↓ IL-1 β ↓ TNF-α ↓ MCP-1	–	↓ NADPH oxidase activity ↓ Nox2 ↔ Nox4 ↑ Activities of complex I and III ↑ Electron-coupling capacity between complexes I and III ↑ Electron-coupling capacity between complexes II and III ↑ I κ B α ↓ NF-κ B ↓ Ang II ↓ ACE expression	Hydrogen-rich saline treatment attenuated left ventricular hypertrophy via reducing oxidative stress, inflammatory process, and angiotensin II, and preserving mitochondrial function in left ventricle	[13]
Male Spontaneously hypertensive rats (SHR) Hypertensive model	Hydrogen-rich saline under 0.4 MPa dissolved in saline for 4 h (0.6 mmol/l)/6 ml/kg/10 wk	IP	↓ HW/BW ↓ LVM/BW Cardiomyocytes were arranged in an orderly manner ↓ CVF	↓ MDA ↑ SOD	–	–	↓ PICP ↓ PIIINP ↓ TIMP ↓ Collagen I ↓ Collagen III ↓ α SMA ↓ Ang II ↓ TGF-β 1 ↓ Smad3 ↓ Smad2/3 ↔ Smad7	Hydrogen-rich saline reduced oxidative stress and improved myocardial collagen content through inhibition of the TGF-β/Smad signaling pathway, leading to a reduction in cardiac hypertrophy	[41]

ACE angiotensin converting enzyme, *AF* atrial fibrillation, *Ang II* angiotensin II, *ANP* atrial natriuretic peptide, *ASC* apoptosis-associated speck-like protein containing a cARd, *ATF* activating transcription factor, *ATP* adenosine triphosphate, *AST* aspartate transaminase, *AW* atial weight, *Bax* apoptosis regulator Bax, *Bcl-2* apoptosis regulator Bcl-2, *BNP* natriuretic peptide, *BW* body weight, *CAT* catalase, *CK-MB* creatinine kinase-MB, *CHOP* the proapoptotic transcriptional factor C/EBP homologous protein, *CVF* collagen volume fraction, *DNA* deoxyribonucleic acid, *Drp1* dynamin-related protein1, *EF* ejection fraction, *ER* endoplasmic reticulum, *FS* fractional shortening, *GPx* glutathione peroxidase, *GSDMD* gasdermin D, *GRP78* glucose-regulated protein 78, *GSH* Glutathione peroxide, *GST* glutathione-S-epoxide transferase, *HC* heart coefficient, *HO1* heme oxygenase 1, *h-FABP* heart-type fatty acid binding protein, *HRS* hydrogen-rich solution, *HR* heart rate, *HW* heart weight, *IL* interleukin, *IP* intraperitoneal, *IRE* inositol-requiring enzyme, *IVSd* intraventricular septum thickness in diastole, *IVSs* intraventricular septum thickness in systole, *JAK/STAT* Janus kinase/signal transducer and activation of transcription signal pathway, *JNK* c-Jun-N-terminal Kinase, *LAD* left anterior descending coronary artery, *LV* left ventricle, *LVAWd* LV anterior wall thickness at end-diastole, *LVEDP* left ventricular end-diastolic pressure, *LV dp/dt* rate of pressure change in left ventricle, *LVSP* LV systolic pressure, *LVDP* LV diastolic/developed pressure, *LVEDd* LV endodiastolic diameter, *LVESd* LV endosystolic diameter, *LVDd* LV internal diameter in diastole, *LVDs* LV internal diameter in systole, *LVPWd* LV posterior wall thickness in diastole, *LVW* left ventricular weight, *IVS* intraventricular septum diameter, *MAP* mean arterial pressure, *MCP* monocyte chemoattractant protein-1, *MDA* malondialdehyde, *MI* myocardial infarction, *MMP* mitochondrial membrane potential, *MPO* myeloperoxidase, *MV* mitral valve, *NADPH* nicotinamide adenine dinucleotide phosphate, *NF-κ B* nuclear factor kappa B, *NLRP3* Nod-like receptor (NLR) family pyrin domain containing protein 3, *NOX* Nox protein, *OGG1* 8-oxoguanine DNA glycosylase, *·OH* hydroxyl radicals, *ONOO–* peroxynitrite, *p* phosphorylation, *PERK* protein kinase RNA-like endoplasmic reticulum kinase, *PICP* procollagen type-I C-terminal peptide, *PGC-1α* peroxisome proliferator-activated receptor-gamma coactivator-1α, *PIIINP* procollagen typer III N-terminal propeptide, *PW* posterior wall thickness, *p38*: 38-kDa protein, *RCR* respiratory control ratio, *ROS* reactive oxygen species, *SBP* systolic pressure, *SD*: Sprague Dawley rat, *SOD* superoxide dismutase, *SGD* serum and glucose deprivation, *Smad* small mothers against decapentaplegic, *SpO2* pulse oximetry, *SS* segment shortening, *T-AOC* total antioxidant capacity, *Tei index* (IVCT + IVRT)/ET, *TGF* transforming growth factor, *TIMP* tissue inhibitors of metalloproteinases, *Tim23* translocase of inner mitochondrial membrane 23, *TNF* tumor necrosis factor, *TnI* troponin I, *Tom20* translocase of outer membrane 20, *Tom40* translocase of the outer mitochondrial membrane 40, troponin I, *TNF-α* tumor-necrosis factor-α, *XBP* X-box binding protein, *4-HNE* 4-hydroxyl-2-nonenal, *8-OHdG* 8-hydroxydeoxyguanosine, *α SMA* alpha-smooth

Table 4 Effects of hydrogen treatment on the heart: reports from ex vivo studies

Study model	Intervention/Dose/Route/Duration	Major findings					Interpretation	References
		Cardiac function/injury marker	Oxidative stress/Anti-oxidant	Inflammation	Cell death	Others		
Female and male Lewis rats (I/R model) Heterotopic heart transplantation using syngeneic grafts from older donors or BN allografts Cold storage 6 h for grafts from syngeneic older Lewis donors or 8 h grafts from allogenic BN	Hydrogen-rich water (1.27 μg/l)/NA/immersed in the water bath at 4 °C/6 or 8 h	↑ Transplant score Less macroscopic myocardial damage Less PMN infiltration ↓ CPK ↓ cTnI	↑ HO1	↓ IL-1 β ↓ IL-6 ↓ TNF-α ↓ ICAM-1 ↓ iNOS ↓ CCL2	-	↑ PGC-1 α ↑ NRF-1 ↑ PPAR-γ ↓ Tissue ATP level	Cold preservation in hydrogen-rich environment ameliorated cardiac injury by inhibiting the infiltration of inflammatory cells and upregulation of pro-inflammatory cytokines, chemokine mRNAs, reduced oxidative stress, and attenuated inflammation	[26]
Male SD rats Heterotopic heart transplantation (I/R model) 6 h/30 min	Hydrogen-rich HTK under 0.4 MPa/3–4 ml/inject via aorta then immersed in 50 ml/6 h						Hydrogen as an additive of HTK solution fortified the preservation efficacy of HTK for cardiac grafts subjected to prolonged cold ischemia by inhibiting cold ischemia-induced up-regulation of oxidative stress, inflammation mediators, and apoptosis in a H_2 concentration dependent manner	[43]
	H_2: HTK = 1:1	↑ LVDP* ↑ LV + dP/dt max ↑ LV-dP/dt max ↑ coronary flow ↓ re-beating time ↓↓↓ myocardial edema and disarrayed	↓ MDA ↓ 8-OHdG ↑ SOD	↓ IL-6 ↓ TNF-α	↓ Apoptotic index ↓ BAX ↓ caspase 3 ↑ Bcl-2	–		
	H_2: HTK = 1:2	↑ LVDP* ↑ LV + dP/dt max ↑ LV-dP/dt max ↔ coronary flow ↓ re-beating time ↓↓ myocardial edema and disarrayed	↓ MDA ↓ 8-OHdG ↑ SOD	↓ IL-6 ↓ TNF-α	↓ Apoptotic index ↓ BAX ↓ caspase 3 ↑ Bcl-2	–		
	H_2: HTK = 1:3	↔ LVDP* ↔ LV + dP/dt max ↔ LV-dP/dt max ↔ coronary flow ↓ re-beating time ↓ myocardial edema and disarrayed	↓ MDA ↓ 8-OHdG ↑ SOD	↓ IL-6 ↓ TNF-α	↓ Apoptotic index ↓ BAX ↓ caspase 3 ↑ Bcl-2	–		

Table 4 (continued)

Study model	Intervention/Dose/Route/Duration	Major findings					Interpretation	References
		Cardiac function/injury marker	Oxidative stress/Anti-oxidant	Inflammation	Cell death	Others		
Male Wistar albino rats I/R model Reperfusion 20 min	Hydrogen-rich water (0.6 mmol/L, PH 7.3)/ NA/perfused after reverse perfusion for 10 min, the treatment was administered at room temperature for 20 min, and reperfusion was performed for 20 min	–	–	↑P-JAK2/ JAK2 ↑P-STAT3/ STAT3	↓ Apoptosis ↑p-AKT/ AKT	↓ 25 DEPs	Hydrogen-rich water decreased apoptosis by up-regulation of the JAK-STAT and PI3K-AKT signaling pathway and alleviated I/R in rats	[44]

Akt protein kinase B, *ATP* adenosine triphosphate, *Bax* apoptosis regulator Bax, *Bcl-2* apoptosis regulator Bcl-2, *CCL* chemokine (C–C motif) ligand, *CPK* creatine phosphokinase, *DEPs* differentially expressed proteins, *HO1* heme oxygenase 1, *HTK* histidine tryptophan ketoglutarate, *ICAM-1* intercellular adhesion molecule 1, *IL* interleukin, *IP* intraperitoneal, *iNOS* inducible nitric oxide synthase, *I/R* ischemia/reperfusion, *JAK/STAT* Janus kinase/signal transducer and activation of transcription signal pathway, *LV dp/dt* rate of pressure change in left ventricle, *LVSP* LV systolic pressure, *LVDP** LV developed pressure (LVSP-LVDP), *LVEDd* LV endodiastolic diameter, *LVESd* LV endosystolic diameter, *LVPWd* LV posterior wall thickness in diastole, *LVW* left ventricular weight, *MDA* malondialdehyde, *NRF-1* nuclear respiratory factor 1, *PGC-1 α*: peroxisome proliferator-activated receptor-gamma coactivator-1 *α*, *PMN*: polymorphonuclear neutrophil, *PPAR-γ*: peroxisome proliferator-activated receptor *γ*, *SD* Sprague Dawley rat, *SOD* superoxide dismutase, *TNF-α* tumor-necrosis factor-*α*, *TnI* troponin I, *8-OHdG* 8-hydroxydeoxyguanosine

five comatose postcardiac arrest patients [45]. The study demonstrated that oxidative stress was reduced while the cytokine levels were unchanged in cardiogenic patients. However, the oxidative stress was unchanged in septic patients, but the cytokine levels were diminished. Nevertheless, the effect of inhaled hydrogen on oxidative stress and cytokines remained inconclusive due to potential methodological weaknesses [45].

Various in vivo and in vitro studies demonstrate hydrogen's ability to reduce inflammation and antiapoptotic properties. A randomized, double-blind, controlled trial showed hydrogen increases antioxidant capacity, thereby reducing inflammatory responses and apoptosis in healthy adults [46].

Because metabolic syndrome remains a serious concern, those patients are at increased risk of developing cardiovascular disease. Hydrogen decreases serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and apo-B levels. Moreover, hydrogen therapy was shown to improve high-density lipoprotein (HDL) function and reduced oxidative stress in patients with metabolic syndrome [47, 48]. All of these clinical studies are comprehensively summarized in Table 5. The potential mechanism of action of molecular hydrogen on selective antioxidants, anti-inflammation, and alleviating cell death are demonstrated in Figs. 1 and 2.

Conclusion and future perspectives

Molecular hydrogen has versatile therapeutic effects due to its small size. It can penetrate the cell membrane and affect metabolism in the body. Molecular hydrogen can be administered via several methods including inhalation, drinking of hydrogen-rich water, injection with hydrogen-rich-saline, and bathing of an organ in a preservation solution. Cumulative evidence from in vivo, in vitro, ex vivo, and clinical studies demonstrated the possible mechanisms underlying the potential benefits of molecular hydrogen, including those increasing antioxidants and decreasing oxidative stress, cell death, metabolism, and inflammation.

For future research, searching for the mechanism of molecular hydrogen to reduce ventricular dilation, decrease wall stress, and reverse adverse cardiac remodeling should be thoroughly investigated. In addition, future clinical studies investigating oxidative stress and inflammatory pathways may provide information to improve the current treatment of various inflammatory diseases, including Kawasaki disease, COVID-19 infection, a multisystem inflammatory syndrome in children or adult (MIS-C or A). Although various in vitro and in vivo models have demonstrated the beneficial effects of molecular hydrogen treatment on the heart,

Table 5 Effects of Hydrogen treatment on the heart: Reports from *clinical* studies

Study model	Intervention		Major findings				Interpretation	References
	Dose/Duration	Route	Cardiac function/injury marker	Oxidative stress/Antioxidant	Inflammation	Others		
20 adult patients with an initial diagnosis of STEMI also undergoing primary percutaneous coronary intervention	H_2 (1.3% H_2 with 26% oxygen)/at emergency room and continued during primary PCI	Inhalation via face mask	↔ cardiac salvage index ↔ ST-segment change ↔ Angiographic myocardial blush score ↔ CK *Hemodynamic measurement (at 6 mo)* ↔ LVEDVi ↔ LVESVi ↑ LVSVi ↑ LVEF	–	–	No adverse event	H_2 gas was feasible, safe and improved the recovery of LV function during reoxygenation after anoxia in the isolated perfused heart	[10]
5 adult patients with post-cardiac arrest syndrome	H_2 (2% H_2 with titrated oxygen)/18 h	Inhalation via using ventilator system					Oxidative stress was reduced, and cytokine levels were unchanged in cardiogenic patients, whereas oxidative stress was unchanged and cytokine levels were diminished in the septic patient. The effect of inhaled H_2 oxidative stress and cytokines remained indefinite due to potential methodological weaknesses	[45]
• Sepsis post-CA ($n=1$)			–	↓ BAP/dROM ↔ 8-OHdG ↔ HEL ↑ LPO	↓ IL-6 ↓ TNF-α	–		
• Cardiogenic post-CA ($n=4$)			–	↓BAP/dROM ↓ 8-OHdG ↓ HEL ($n=1$) ↔ LPO	↔ IL-6 ($n=3$) ↔ TNF-α	–		
38 healthy adults	Hydrogen-rich water/dose: 1.5L/d/4 wk	Oral		↔ BAP ↔ dROM ↔ 8-OHdG	↓ IL-6	↓ Apoptotic cells (Annexin V + DAPI+) ↓ CD14 ↓ NF-κ B	Hydrogen-rich saline increases antioxidant capacity thereby reducing inflammatory responses and apoptosis in healthy adults	[46]

Table 5 (continued)

Study model	Intervention		Major findings				Interpretation	References
	Dose/Duration	Route	Cardiac function/injury marker	Oxidative stress/Antioxidant	Inflammation	Others		
• Age ≥ 30 y				↓ BAP ↓ dROM ↓ 8-OHdG				
20 adult patients with potential metabolic syndrome	Hydrogen-rich water/dose: 1.5–2 L/d/8 wk			↑ SOD	↓ TBARS in urine	↑ HDL-C ↓ TC/HDL-C	Hydrogen-rich saline decreases serum TC/HDL-C levels, improves HDL-C level, and reduces oxidative stress in patients with potential metabolic syndrome	[47]
20 adult patients with potential metabolic syndrome	Hydrogen-rich water/dose: 0.9–1 L/d/10 wk			↑ SOD	↓ TNF-*α*	↓ TC ↓ LDL-C ↓ apo B 100 ↓ apo E ↑ HDL function	Hydrogen-rich saline decreases serum TC and LDL-C and apo B levels, improves HDL function, and reduces oxidative stress in patients with potential metabolic syndrome	[48]

apo apolipoprotein, *BAP* biological antioxidant potential, *CA* cardiac arrest syndrome, *CK* creatinine kinase, *LV* left ventricle, *HDL-C* high-density lipoprotein cholesterol, *dROMs* derivatives of reactive oxygen metabolites, *HEL* N-hexanoyl-lysine, *IL* interleukin, *LPO* lipid hydroperoxide, *LDL-C* low-density lipoprotein cholesterol, *LVEDVi* LV end-diastolic volume index, *LVESVi* LV end-systolic volume index, *LVSVi* LV stroke volume index, *LVEF* LV ejection fraction, *MI* myocardial infarction, *PCI* percutaneous coronary intervention, *SOD* superoxide dismutase, *STEMI* ST-elevated MI, *TBARS* thiobarbituric acid reactive substances, *TC* total cholesterol, *TNF-α* tumor-necrosis factor-*α*

clinical investigations are still limited. Future large-scale randomized control trials are needed to determine the crucial clinical impact of using hydrogen as a therapy, and to verify the efficacy and safety of clinical interventions with molecular hydrogen to warrant its use and to improve medical treatment in this field.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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