Minireview

Protective effects of molecular hydrogen on lung injury from lung transplantation

Lini Quan[1], Bin Zheng[2] and Huacheng Zhou[2]

[1]Department of Anesthesiology, Second Affiliated Hospital of Harbin Medical University, Harbin 150086, China; [2]Department of Anesthesiology, Fourth Affiliated Hospital of Harbin Medical University, Harbin 150001, China
Corresponding author: Huacheng Zhou. Email: zhouhuacheng@163.com

Impact statement

This review summarizes currently available data regarding the protective role of hydrogen in lung injuries caused by lung transplantation, provides an outline of recent advances in the use of hydrogen as a therapeutic medical gas for lung transplantation, and elucidates possible mechanisms underlying the protective effect of hydrogen on lung grafts. This review of experiments on animals will lay the foundation for the application prospects and clinical value of hydrogen during lung transplantation.

Abstract

Lung grafts may experience multiple injuries during lung transplantation, such as warm ischaemia, cold ischaemia, and reperfusion injury. These injuries all contribute to primary graft dysfunction, which is a major cause of morbidity and mortality after lung transplantation. As a potential selective antioxidant, hydrogen molecule (H_2) protects against post-transplant complications in animal models of multiple organ transplantation. Herein, the authors review the current literature regarding the effects of H_2 on lung injury from lung transplantation. The reviewed studies showed that H_2 improved the outcomes of lung transplantation by decreasing oxidative stress and inflammation at the donor and recipient phases. H_2 is primarily administered via inhalation, drinking hydrogen-rich water, hydrogen-rich saline injection, or a hydrogen-rich water bath. H_2 favorably modulates signal transduction and gene expression, resulting in the suppression of pro-inflammatory cytokines and excess reactive oxygen species production. Although H_2 appears to be a physiological regulatory molecule with antioxidant, anti-inflammatory and anti-apoptotic properties, its exact mechanisms of action remain elusive. Taken together, accumulating experimental evidence indicates that H_2 can significantly alleviate transplantation-related lung injury, mainly via inhibition of inflammatory cytokine secretion and reduction in oxidative stress through several underlying mechanisms. Further animal experiments and preliminary human clinical trials will lay the foundation for the use of H_2 as a treatment in the clinic.

Keywords: Hydrogen, lung transplantation, ischaemia–reperfusion injury, inflammation, oxidative stress, antioxidant

Experimental Biology and Medicine **2021; 246: 1410–1418. DOI: 10.1177/15353702211007084**

Introduction

Lung transplantation (LTx) is the final approach to promote survival for patients with end-stage pulmonary disease. However, the outcomes of LTx are inferior to those of other solid organ transplantations.[1] Primary graft dysfunction (PGD) is a major cause of morbidity and mortality after LTx.[2] PGD can increase the length of mechanical ventilation, prolong hospital stays, and induce bronchiolitis obliterans syndrome.[3] Multiple mechanisms contribute to the pathogenesis of PGD, including ischaemia–reperfusion (I/R) injury, epithelial cell death, endothelial cell dysfunction, innate immune activation, oxidative stress, and inflammatory cytokine and chemokine release.[2] I/R injury is generally considered the major contributor to PGD and may also aggravate rejection. Both I/R injury and PGD are associated with the development and progression of chronic lung allograft dysfunction.[4] In addition to I/R injury, complex interactions of donor injury before transplantation, such as preceding brain death, cardiocirculatory death, and pre-engraftment lung management, also impair the status of donor lungs.[5]

In 2007, Ohsawa *et al.* discovered that hydrogen molecule (H_2) has antioxidative and anti-apoptotic properties, which protect the brain against I/R injury and stroke by selectively neutralizing hydroxyl radicals.[6] Since then, hydrogen gas has emerged at the forefront of therapeutic medical gas research. Accumulating evidence has proved that molecular hydrogen exerts cytoprotective effect by its

Copyright © 2021 by the Society for Experimental Biology and Medicine

antioxidative, anti-inflammatory and anti-apoptotic properties. In recent years, growing experimental evidence showed that hydrogen can remarkably ameliorate transplantation-related lung I/R injury. Hydrogen inhalation for donors or recipients has been shown to mitigate lung injury and ameliorated lung dysfunction after LTx. Preconditioning lung donors with hydrogen during preservation could also decrease post-transplant lung injury.

Due to its reductive properties, hydrogen can be applied as an antioxidant to selectively eliminate toxic reactive oxygen species (ROS) without affecting the normal physiological function of ROS.[6] Due to its ability to rapidly diffuse across the membranes of cells and organelles, hydrogen can react with cytotoxic ROS in subcellular structures and thus protect against oxidative damage. At present, the main routes of hydrogen administration include inhalation, drinking or injection of hydrogen-dissolved solution, and hydrogen bath. With unique structural characteristics, the lung allows hydrogen to exchange gas without entering the blood circulation and thus enables hydrogen to exert protective effects during the donor preservation period.

The protective effect of hydrogen on donor lung injury

Donor lungs may experience numerous injurious events prior to LTx, including injury before organ procurement, such as donor mechanical ventilation, warm ischaemia phase (WIP) injury from cardiocirculatory death, brain death, and cold ischaemia injury during organ preservation.[7–9]

Application of hydrogen during WIP protects lung grafts from I/R injury

The donation of lungs following brain death and cardiocirculatory death is the main source to expand the lung donor pool. However, the inevitable occurrence of WIP can activate the immune system in donors and aggravate I/R injury, which increases the risk of PGD.[10] In a rat model of LTx, we showed that 3% hydrogen gas ventilation during WIP improved post-transplantation lung function, as determined by improved partial pressure of arterial oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio and static compliance of the lung grafts. Hydrogen preconditioning alleviated cardiac death lung graft I/R injury by reducing inflammatory mediator upregulation, demonstrated by decreased myeloperoxidase (MPO) activity, lower serum interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels, and elevated IL-10 level. Hydrogen was also shown to attenuate oxidative stress evidenced by increased superoxide dismutase (SOD) activity and a decreased malondialdehyde (MDA) level, and to decrease the alveolar epithelia cell apoptosis in lung grafts.[11] Brain death initiates a cascade of events such as hemodynamic, hormonal, metabolic, and inflammatory changes, all of which can impact the quality and immune activation of potential donor organs. Previous studies on I/R injury showed a confounding issue: the function of otherwise healthy donor lungs from brain dead donors is impaired.[12] In a rat model of LTx wherein both brain dead donors and recipients received 2% hydrogen ventilation, we found that hydrogen treatment could decrease the IL-8, TNF-α and intracellular adhesion molecule (ICAM)-1 levels, increase SOD activity and reduce MDA levels, and mitigate apoptosis in lung grafts, leading to improved PaO_2/FiO_2 ratio, static compliance, and graft histology.[13]

In 2011, Kawamura *et al.* discovered in a rat model of LTx that the ventilation of the donor with 2% hydrogen gas prior to procurement can attenuate the upregulation of the mRNA of pro-inflammatory cytokines TNF-α, IL-1β, and ICAM-1, increase the levels of the anti-apoptotic protein B-cell lymphoma-2 (Bcl-2), and decrease the levels of the proapoptotic molecules Bcl-2-associated X (Bax)-protein significantly. Lower lung injury scores and increased PaO_2 in lung allografts after reperfusion demonstrated that hydrogen exposure protected lung grafts from I/R injury and mitigated lung dysfunction resulting from LTx.[14] In 2012, Tanaka *et al.* found that ventilation with 2% hydrogen in donor rats could significantly upregulate 182 target gene expressions, and downregulate 47 target gene expressions by gene array analysis. Further investigation found that preloading hydrogen into the lung significantly upregulated four lung surfactant-related mRNAs, especially Clara cell protein (CCP) 16, a protein major produced by the non-ciliated cells of the tracheobronchial epithelial tree with anti-inflammatory and antioxidant properties, the mRNA levels of four kinds of adenosine triphosphate (ATP) synthase genes, and stress-response gene heat shock protein (HSP) A5, which encodes a member of the HSP70 protein family in the lung grafts, as detected by real-time RT-PCR. The protein levels of CCP16 and HSP70, and the ATP levels in the lung graft tissue were also increased in the presence of hydrogen. The changes in these molecules may underlie the protective effects of hydrogen against I/R injury during LTx.[15]

Preloading hydrogen during organ preservation ameliorates I/R injury of lung grafts

In a rat model of LTx, we found that donor lung inflation with 3% hydrogen during the cold ischaemic period could reduce graft MPO activity and serum IL-8 and TNF-α levels, increase PaO_2/FiO_2 and pulmonary venous oxygen tension (PvO_2)/FiO_2, resulting in alleviated lung graft injury and improved function.[16] In 2016, we demonstrated that inflation with CO or H_2 protected against I/R injury in a rat lung transplantation model, and this effect was enhanced by combined CO and H_2 treatment.[17] According to the results of our recent study, the application of hydrogen gas during cold storage attenuated the inflammatory response in an LTx model in pulmonary microvascular endothelial cells, and this effect may be achieved by inhibition of the p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) pathways.[18] Kayawake *et al.* discovered that hydrogen-rich preservation solution increased PaO_2 and the pulmonary dynamic compliance, decreased wet-to-dry lung weight ratio, reduced

neutrophil infiltration and the expression of IL-1β mRNA in lung grafts in a canine left LTx model.[19] Another study by Saito *et al.* showed the similar results in a rat LTx model that hydrogen-rich preservation solution could decrease leukocytes adhering to the endothelium and the expression of IL-1β mRNA, reduced the level of 8-hydroxydeoxyguanosine, an oxidation marker of DNA.[20] Immersing the lungs in hydrogen-rich saline (HRS) could decrease pro-inflammatory cytokine levels (IL-1β and IL-6) and high mobility group box-1 expression in a rat model of lung I/R injury.[21] These evidence suggested that a hydrogen-rich preservation solution might exert protective effects through antioxidant and anti-inflammatory effects. Donor preservation using a hydrogen-rich solution appears to be a promising approach to managing lung I/R injury.

Hydrogen gas inhalation during EVLP exerts beneficial effects on lung grafts

Normothermic *ex vivo* lung perfusion (EVLP) is a system that can perfuse and ventilate a retrieved donor lung through an *ex vivo* circuit. EVLP could provide an opportunity to reassess the viability of the injured donor lung before LTx. Noda *et al.* found that administering 2% hydrogen through the EVLP circuit attenuated pro-inflammatory responses, decreased lactic production caused by EVLP, downregulated hypoxia inducible factor-1 expression, and promoted mitochondrial biogenesis. Additionally, hydrogen increased the expression of heme oxygenase-1 (HO-1), a well-known key antioxidant enzyme, in the lung grafts.[22] These effects suggested that hydrogen-supplemented preconditioning contributed to better post-transplant outcomes in the recipients. Haam *et al.* found that hydrogen gas inhalation during EVLP could improve the lung compliance, peak airway pressure, and oxygen capacity (left atrium perfusate PO_2—pulmonary artery perfusate PO_2/FiO_2 ratio) of lung grafts in a pig LTx model. Hydrogen gas exerted antioxidant effect in I/R injured lung grafts, as demonstrated by elevated protein expression of SOD and HO-1. The lower expression of IL-6 and NOD-like receptor protein 3 (NLRP3), a component of inflammasome, higher expression level of anti-inflammatory cytokine IL-10, as well as the decreased number of apoptosis cells after the hydrogen treatment indicated that the mechanisms underlying the beneficial effects of hydrogen involve the action of anti-inflammation and anti-apoptosis.[23,24]

Administrating hydrogen to recipients after LTx mitigates the I/R injury in lung grafts

In a rat left orthotopic LTx model, Kawamura *et al.* found that ventilation with 2% hydrogen during surgery and for 1 h after reperfusion alleviated graft dysfunction. The reduced upregulation of mRNAs for ICAM-1, TNF-α, IL-1β, and IL-6 after hydrogen treatment showed that hydrogen ameliorated inflammatory responses associated with I/R injury. Hydrogen mitigated I/R-induced graft apoptosis, as determined by upregulation of Bcl-2 and B-cell lymphoma-extra large (Bcl-xL) mRNAs and protein as well as reduced activated caspase-8 levels.[25] In a rabbit I/R injury model, HRS applied intraperitoneally before reperfusion decreased neutrophil infiltration, reduced the elevation of TNF-a and IL-8. Besides, HRS treatment reduced MDA contents and increased SOD activities at the end of reperfusion. Based on these findings, they demonstrated that the protective effects of HRS in attenuating lung I/R injury seemed to be associated with mitigating inflammation and oxidative damage.[26]

Hydrogen had an active effect on immunity. Ozeki *et al.* recently discovered that hydrogen-saturated water administration after transplantation suppressed the development of mid-term obliterative airway disease via antioxidant and anti-inflammatory mechanisms in a mouse heterotopically tracheal allograft model, and hydrogen could activate regulatory T-cells. Hydrogen water reduced the level of IL-6 and increased that of forkhead box P3 transcription factor and CD4/CD3, suggesting that hydrogen enhanced regulatory T cell activity.[27] Noda *et al.* found that drinking a hydrogen-rich solution inhibited the proliferation of T cells and the production of IFN-γ and IL-2, and hydrogen was remarkably effective in prolonging graft survival and reducing the proliferation of intimal mast cells.[28]

Different ways of molecular hydrogen administration and their effects on lung grafts after LTx are summarized in Table 1.

Possible mechanisms of the protection effect of hydrogen

Antioxidant effects of molecular hydrogen

Hydrogen may protect against transplant-induced oxidative stress injury both by hydrogen's free radical scavenging activities, and by inducing nuclear factor E2-related factor 2 (Nrf2)-dependent protective signaling pathways.

Free radicals are produced primarily in the respiratory chain, phagocytosis, prostaglandin synthesis, and the cytochrome P450 system.[29] Excessive ROS can react with multiple macromolecules, causing deleterious consequences. Hydroxyl radicals are the most toxic and reactive radicals and are biologically formed by the reaction of O_2^- with H_2O_2 through the Fenton reaction.[30] Ohsawa *et al.* demonstrated that hydrogen directly reacts with hydroxyl radicals in a cell-free system and reduces hydroxyl radicals in cell culture using the method of spin-trapping by 5,5-dimethyl-1-pyrroline N-oxide, selectively scavenging oxygen hydroxyl radicals.[6] A large amount of evidence has demonstrated the radical scavenging capacity of molecular hydrogen.[31] Fang *et al.* showed that hydrogen treatment significantly reduced the intracellular levels of ROS and MDA in both vitiligo specimens and hydrogen peroxide-treated melanocytes *in vitro*.[32] Subsequent studies showed that hydrogen mitigates oxidative stress in animal I/R injury models, which was supported by decreased lipid peroxidation and MDA production.[11,33,34]

The Nrf2-antioxidant response element pathway is considered an essential pathway for protection against oxidative stress for its transcriptionally regulating numerous antioxidative and cytoprotective proteins. Hydrogen gas

Table 1. Different ways of molecular hydrogen administration and their effects in lung transplantation.

		Effects			
Form	**Ways of administration**	**Anti-oxidation**	**Anti-inflammation**	**Anti-apoptosis**	**Improvement on post-transplant graft functional parameters**
Hydrogen gas	Ventilation for donors[11,14,15]	HO-1 mRNA and protein ↑[14] SOD activity ↑[11] MDA levels↓[11]	ICAM-1, TNF-α, IL-1β mRNA ↓[14] MPO activity ↓[11] Serum IL-6 and TNF-α levels ↓[11] Nuclear NF-κB protein ↓[11] Phosphorylated p38, ERK1/2 and JNK protein ↓[15]	Alveolar epitdelial cell apoptosis ↓[11,14] Bcl-2 protein ↑[14] Bax protein ↓[14]	PaO_2/FiO_2 ↑[11] Static compliance of tde lung grafts ↑[11]
	Ventilation for recipients[25]	MDA concentration ↓ 4-HNE ↓ Alveolar macrophage infiltration ↓	ICAM-1, TNF-α, IL-1β, and IL-6 mRNA ↓	Alveolar epitdelial cell apoptosis ↓ Bcl-2 mRNA and protein ↑ Bcl-Xl mRNA and protein ↑ Bax mRNA and protein ↓ Caspase-8 protein ↓	PO_2 in tde graft pulmonary vein ↑ PCO_2 in tde graft pulmonary vein ↓
	Ventilation for donors and recipients[13]	SOD activity ↑ MDA levels ↓	ICAM-1 positive cells ↓ Serum IL-8 and TNF-α ↓	Alveolar epitdelial cells apoptosis ↓ tde number of caspase-3 positive cells ↓	PaO_2/FiO_2 ↑
	Lung inflation during cold preservation[16,17]	MDA activity ↓ SOD activity ↑	Serum IL-8 and TNF-α ↓ MPO activity ↓	Alveolar epitdelial cells apoptosis ↓ tde number of caspase-3 positive cells ↓	PaO_2/FiO_2 ↑
	Ventilation during EVLP[22–24]	HO-1 and SOD protein ↑[24] HO-1 mRNA ↑[22]	NLRP3 and IL-6, protein ↓ IL-10 protein ↑[24] IL-1β, IL-6, IL-8, and TNF-α concentration ↓[23] IL-6, IL-1β, and TNF-α mRNAs ↓[22] HIF-1-α mRNA ↓22 Phosphorylated p38, ERK1/2 and JNK protein ↓[23]	Apoptotic cells ↓[24]	PaO_2/FiO_2 ↑[22] Pulmonary vascular resistance ↓[22–24] Peak airway pressure ↓[23,24] Oxygen capacity ↑[24] Lung compliance ↑[22,24]
Hydrogen-rich solution	Lung grafts flushing and immerging during cold preservation[19,20]	8-OHdG ↓[20] Nrf2 and HO-1 mRNA ↑[20] Leukocyte adhesion on the endothelium ↓ [20]	IL-1β mRNA ↓[19,20] TNF-α mRNA ↓[20] Neutrophil infiltration ↓[20]	Apoptotic cells ↓[19]	PaO_2 ↑[19,20] $PaCO_2$ ↓[19] Pulmonary dynamic compliance ↑[19,20] Peak airway pressure ↓[20]

MPO: myeloperoxidase; MDA: malondialdehyde; SOD: superoxide dismutase; IL: interleukin; TNF-α: tumor necrosis factor-α; 8-OHdG: 8-hydroxydeoxyguanosine; 4-HNE: 4-hydroxy-2-nonenal; PO_2: arterial partial pressure of oxygen; $PaCO_2$: arterial partial pressure of carbon dioxide; PaO_2: partial pressure of arterial oxygen; FiO_2: fraction of inspired oxygen; HIF: hypoxia-inducible factor; Nrf2: nuclear factor E2-associated factor 2; HO-1: heme oxygenase-1; ICAM-1: intracellular adhesion molecule-1; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-associated X; Bcl-xL: B-cell lymphoma-extra large; NF-κB: nuclear factor-kappa B; ERK: extracellular signal-regulated kinase; JNK: jun N-terminal kinase.

can ameliorate oxidative stress through induction of Nrf2-dependent genes, such as HO-1 and SOD. The potential mechanism of molecular hydrogen activation of Nrf2 will be discussed in more detail later.

Hydrogen reduces inflammation

TNF-α, IL-1β and IL-6 are the most important inflammatory factors that mediate the inflammatory response and play an important role in the cascade of inflammation. Hydrogen can directly reduce the mRNA expression of inflammatory factors such as TNF-α, IL-1β, IL-6, and ICAM-1, inhibit the mRNA production of chemokines, such as C-C motif ligand 2, and reduce leukocyte accumulation.[21,35] Hydrogen can also reduce the activity of MPO, indicating a decrease in the accumulation of neutrophils.[13,36] Zhai *et al.* found that HRS could reduce neutrophil accumulation as evidenced by reduced MPO activity in rat cecal ligation and puncture-induced sepsis-associated acute lung injury models. In addition, the decrease in the levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, in the lung also suggested that HRS could ameliorate pulmonary inflammatory responses.[35]

Hydrogen inhibits apoptotic cascades

Number of animal experiments have showed that hydrogen can decrease apoptosis of alveolar epithelial cells and pulmonary microvascular endothelial cells in lung grafts.[11,16,18] The anti-apoptotic abilities of hydrogen were at least partially attributed to the regulation of the apoptosis-related genes such as Bcl-2, Bcl-xL, and Bax. Sequential activation of caspases, which plays a central role in the process of cellular apoptosis, can also be inhibited by hydrogen molecular. The number of apoptotic pulmonary epithelial cells was reduced after hydrogen treatment. Accordingly, hydrogen treatment was found to increase the levels of the anti-apoptotic protein Bcl-2 and decrease the levels of the proapoptotic Bax protein significantly in the lung graft of rats.[14] Hydrogen gas inhalation was shown to upregulate Bcl-2 and Bcl-xL mRNAs and protein and inhibit the upregulation of Bax mRNA and protein in lung grafts of rats. Moreover, hydrogen treatment was observed to reduce activated caspase-8 levels.[25] Zhang *et al.* showed that hydrogen can significantly upregulate the expression levels of Bcl-2 and Bcl-xl in a rat model of endotoxin-induced lung injury, while downregulate the expression of the apoptotic genes caspase-3, caspase-8, and Bax in the lung grafts.[37] Hydrogen inhalation was also reported to increase the expression of Bcl-2 and reduce ventilator-induced lung injury (VILI)-induced expression of the Bax protein in lungs.[38] Qiu *et al*. showed that lipopolysaccharide (LPS) resulted in an upregulation of Bax protein and hydrogen inhalation inhibited overactivity of Bax protein.[39] These influences of hydrogen on apoptosis-related genes can reduce the secretion of proteases, protect the integrity of cells from damage, and inhibit the initiation of apoptosis programs.[13]

Hydrogen protects mitochondrial structure and function in I/R injury

Mitochondria is the major site of generation of intracellular ROS as well as the target of ROS. I/R results in the production of mitochondrial-derived ROS, collapse of the mitochondrial membrane potential, opening of the mitochondrial permeability transition pore (mPTP), an influx of calcium into the mitochondria and release of cytochrome c.[40] Hydrogen molecules can reach subcellular structures, such as the nucleus and mitochondria. Ordinary antioxidants do not effectively target mitochondrial ROS, but hydrogen molecules can selectively scavenge $^{-}$OH and peroxynitrite anions as detected by fluorescent probes and electron paramagnetic resonance spectroscopy.[41] As a selective antioxidant, hydrogen molecules have the capacity to reduce mitochondrial oxidative stress, and thus protect against mitochondrial dysfunction and inhibit mitochondria-mediated apoptosis.

Iuchi *et al.* found that molecular hydrogen restored the decline of oxidoreductase activity and mitigate the loss of mitochondrial membrane potential induced by *tert*-butyl hydroperoxide (a free radical inducer) in a human acute monocytic leukaemia cell line, indicating that hydrogen suppressed oxidative stress-induced mitochondrial injury.[42] Increasing of the coenzyme Q9 concentration by hydrogen-rich water administration can stimulate the transfer of electrons from complex I and complex II to complex III, and increase the level of ATP production through mitochondrial oxidative phosphorylation in rat heart mitochondria.[43] Hydrogen treatment was also associated with increased graft ATP levels and increased activity of the enzymes in the mitochondrial respiratory chain.[28] Sobue *et al.* found that hydrogen molecules activated the expression of sets of genes regulated by histone H3K27 methylation status through H3K27 demethylase, which significantly increased the expression of mitochondrial HSP and then played a role in mitochondrial repair.[44] Cui *et al.* showed that after HRS treatment, the mitochondria were less swollen and exhibited membranes integrity with higher mitochondrial membrane potential in cerebral I/R rats during reperfusion. HRS was also shown to significantly attenuate mPTP and reduce the release of pro-apoptotic factor cytochrome c from mitochondria following cerebral I/R.[45] HRS improves the bile duct ligation-induced decline in mitochondrial respiratory function and the integrity of the respiratory chain as assessed by the adenosine diphosphate (ADP) to oxygen ratio and the mitochondrial respiratory control ratio (respiration rate after the addition of 1 mM ADP/oxygen consumption rate after the complete phosphorylation of ADP). HRS can also inhibit the depletion of ATP levels and mitochondrial cytochrome c release in hepatocytes of mice with obstructive jaundice.[46]

Hydrogen attenuates I/R injury by suppressing endoplasmic reticulum stress

Oxidative stress, calcium overload, and inflammation in the process of I/R injury can activate endoplasmic reticulum stress (ERS), and in turn, the cell dysfunction and

apoptosis induced by excessive ERS aggravate I/R injury.[47] ERS activates signaling pathways, such as the unfolded protein response, ER overload response, and caspase-12-mediated apoptotic pathways. The activation of ERS caused caspase-12 to translocate from the ER to the cytoplasm, cleaved caspase-9, activated caspase-3, and finally led to apoptosis. HRS has been reported to mitigate ERS and ERS induced apoptosis resulting from I/R in multiple organs including myocardium,[48] intestinal,[49] liver,[50,51] and brain,[52] as revealed by down-regulated mRNA and protein levels of the pivotal proteins involved in ERS, such as glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP). According to the results of research by Sun *et al*., HRS treatment could alleviate the elevated GRP78, CHOP and X-box binding protein-1mRNA expression induced by hyperoxia exposure, hinting that hydrogen could significantly reduce hyperoxic acute lung injury in rats by inhibiting the elevation in ERS.[53] The mechanisms mediate the reduction in ERS by hydrogen might related to cross-talk between autophagy and ERS. Hydrogen significantly reduced the expression of ERS-related protein and increased the autophagy-related protein expression in septic mice. Moreover, treatment with the inducer of ERS or the inhibitor of autophagy abrogated these effects of hydrogen. These results suggested that hydrogen alleviated ER stress via the autophagy pathway in sepsis.[54] However, Gao *et al.* reported that hydrogen exerted protective effect on myocardial I/R injury in rats by attenuating ERS and down-regulating autophagy.[48]

Hydrogen modulates intracellular signaling pathways

Nrf2/HO-1

Nuclear factor E2-associated factor 2 (Nrf2) is considered a key regulator for maintaining the redox balance and controlling the initiation of the transcriptional expression of downstream antioxidant enzymes. The activation of the Nrf2 pathway initiates antioxidant defense through a variety of mechanisms, including SOD-induced catabolism of superoxide and peroxide, regeneration of antioxidant cofactors and proteins, and increase in redox transport.[55]

The mechanism of antioxidant effects of molecular hydrogen was shown to involve in the activation of Nrf2 signaling pathway in lung injury induced by hyperoxia,[36] seawater instillation,[56] sepsis,[57] and paraquat-induced lung fibroblast injury *in vitro*.[58] Hydrogen was shown to increase pulmonary HO-1 protein and mRNA expression, as well as HO-1 activity in the lungs of rats exposed to hyperoxia. Moreover, Nrf2 knockout mice showed little improvement with hydrogen treatment, suggesting that the beneficial effect of molecular hydrogen is Nrf2-dependent.[36] Hydrogen was reported to upregulate the HO-1 mRNA expression and protein level in the allografts, which might contribute to the protective effects of hydrogen.[14] Kelch-like ECH-associated protein 1 (Keap1) located in cytoplasm negatively regulated Nrf2 by binding the N-terminal Neh2 domain of Nrf2. Upon exposure to ROS, Nrf2 dissociates from Keap1 and is translocated into the nucleus, resulting in an increased expression of antioxidant genes. Lu *et al.* showed in a rat model of cyclosporine A-induced nephrotoxicity that HRS administration decreased the mRNA and protein expression of Keap1 without affecting the levels of Nrf2 mRNA. Meanwhile, HRS significantly decreased the protein expression of cytosolic Nrf2, while increased in the expression of nuclear Nrf2, but the total expression of Nrf2 did not increase in rats treated with.[59] These results indicated that hydrogen protects against oxidative stress injuries via the activation of the Keap1/Nrf2 signaling pathway.

NF-κB

NF-κB is a pleiotropic oxidant-sensitive transcription factor that exists in the cytosol in an inactive form complexed to an inhibitory kappa B (I-κB) monomer. Various stimuli, including ischaemia, hypoxia, free radicals, cytokines, and LPS, activate NF-κB by inducing the phosphorylation of I-κB. Zhang *et al.* found that hydrogen treatment obviously reduced nuclear NF-κB protein expression in the lung graft of rats, indicating hydrogen may exerted anti-inflammatory and anti-oxidative effects against lung I/R injury via the NF-κB signaling pathway.[11] Kohama *et al.* showed that hydrogen inhalation could inhibit inflammation in hemorrhagic shock and resuscitation-induced acute lung injury rats by inhibiting the NF-κB pathway.[60] Xie *et al.* found that lung inflammation and apoptosis were decreased by hydrogen treatment via inhibiting the NF-κB activity in a rat model of LPS-induced lung injury.[61] In contrast, Huang *et al.* reported that hydrogen inhalation resulted in early NF-κB activation in the lungs of mice during VILI as determined by the DNA binding activity of NF-κB, and inhibition of NF-κB reversed the protective effects of hydrogen. These results suggested that the cytoprotective effects of hydrogen against apoptotic and inflammatory may in part depend on the activation of NF-κB during VILI.[38]

MAPK

MAPK primarily regulates transcription factors through phosphorylation, including p38, extracellular signal-regulated kinase (ERK), and jun N-terminal kinase (JNK). The activation of p38MAPK is the trigger of inflammation-induced lung injury. The mRNA of dual specificity phosphatase1, a dual specificity phosphatase that decreases MAPK phosphorylation/activation in the lung grafts, were upregulated by hydrogen, which resulted in reduced protein levels of p38 MAPK, ERK1/2, and JNK in the allografts.[15] We found that the anti-inflammatory effect of hydrogen may be due to a decrease in secretion of pro-inflammatory cytokines by inhibiting the activation of the p38 MAPK pathway in pulmonary microvascular endothelial cells.[18] Zhai *et al.* showed that HRS peritoneal injection inhibited the activation and phosphorylation of p38MAPK and NF-κB.[35] As reported by Zhang *et al*., saturated hydrogen saline may attenuate LPS-induced acute lung dysfunction in rats by reducing inflammation, autophagy, and apoptosis in a mechanism involving the p38 MAPK signalling pathway.[37] Qiu *et al.* found that hydrogen can inhibit

the production of ROS in lung tissue caused by endotoxins and inhibit the activation of p-JNK.[39] Bai *et al.* proved that saturated hydrogen water could reduce the occurrence of the inflammatory cascade and organ dysfunction by inhibiting the ERK1/2 pathway.[52] Intraperitoneal injection of HRS inhibited LPS-induced activation of p38 MAPK and JNK, which is associated with LPS-induced downregulation of aquaporin-1 (AQP-1) andaquaporin-5 (AQP-5).[62]

AQP-1 and AQP-5

The aquaporins (AQPs) are plasma membrane water-transporting proteins. AQP has an important role in transporting water across the lung microvascular endothelium as well as across the lung epithelia.[63] In mice, lung AQP expression seems to be related to the agent applied to induce lung injury. I/R of lung tissue increased AQP-1 expression[64] and reduced AQP-5 expression,[65] while LPS reduced the expression of AQP-5 and AQP-1.[66] In a rat model of LPS-induced lung injury, hydrogen improved the alveolar-arterial oxygen partial pressure difference (A-aDO2) and protected pulmonary epithelial barrier function by increasing AQP-1 expression and decreasing extravascular lung water (EVLW). A significant negative correlation between AQP-1 expression and EVLW was observed.[67] Tao *et al.* revealed that HRS can export fluid in alveoli, lung tissue spaces, and pulmonary vessels by upregulating AQP-1 and AQP-5 and thus attenuate LPS-induced pulmonary edema.[62]

Conclusions

Taken together, accumulating evidence from animal studies suggests that the use of hydrogen can mitigate the injury of lung graft resulting from LTx and improve the lung graft function. Hydrogen exerts many biological effects, including anti-oxidative stress, anti-inflammation, anti-apoptosis on lung grafts by indirectly regulating signal transduction and gene expression, each of which involve multiple several cytoprotective signaling pathways and crosstalk. As a small molecule gas, hydrogen can penetrate biological membranes to exert these protective effects on cells and organelles. Due to its characteristics of safety, low price, easy accessibility, and lack of accumulation, hydrogen has promising prospects for clinical application. Research on hydrogen treatment for transplantation-induced lung injury is still in the preclinical stage in small animal models, such as rats and rabbits, and the mechanism of the protective effect of hydrogen on lung injury after LTx is still unclear. The preliminary clinical application of hydrogen for human requires a long-term exploration process.

AUTHORS' CONTRIBUTIONS

QL conceived and designed the research, acquired the data, and drafted the manuscript; ZB participated in data collection, figure assessment, and analysis of the manuscript; ZH conceived and designed the research, handled funding, supervision and correspondence, and made critical revisions of the manuscript for important intellectual content.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by National Natural Science Foundation of China (Grant No. 81570088).

ORCID iD

Huacheng Zhou https://orcid.org/0000-0003-1470-5434

REFERENCES

1. Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D Jr, Hsich E, Meiser B, Potena L, Robinson A, Rossano JW, Sadavarte A, Singh TP, Zuckermann A, Stehlik J, International Society for Heart and Lung transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report – 2019; focus theme: donor and recipient size match. *J Heart Lung Transplant* 2019;**38**:1056–66
2. Porteous MK, Lee JC. Primary graft dysfunction after lung transplantation. *Clin Chest Med* 2017;**38**:641–54
3. Diamond JM, Arcasoy S, Kennedy CC, Eberlein M, Singer JP, Patterson GM, Edelman JD, Dhillon G, Pena T, Kawut SM, Lee JC, Girgis R, Dark J, Thabut G. Report of the International Society for Heart and Lung Transplantation Working Group on Primary Lung Graft Dysfunction, part II: epidemiology, risk factors, and outcomes – a 2016 Consensus Group statement of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2017;**36**:1104–13
4. Whitson BA, Prekker ME, Herrington CS, Whelan TP, Radosevich DM, Hertz MI, Dahlberg PS. Primary graft dysfunction and long-term pulmonary function after lung transplantation. *J Heart Lung Transplant* 2007;**26**:1004–11
5. Snell GI, Levvey BJ, Levin K, Paraskeva M, Westall G. Donation after brain death versus donation after circulatory death: Lung donor management issues. *Semin Respir Crit Care Med* 2018;**39**:138–47
6. Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med* 2007;**13**:688–94
7. Iskender I, Cypel M, Martinu T, Chen M, Sakamoto J, Kim H, Yu K, Lin H, Guan Z, Hashimoto K, Waddell TK, Liu M, Keshavjee S. Effects of warm versus cold ischemic donor lung preservation on the underlying mechanisms of injuries during ischemia and reperfusion. *Transplantation* 2018;**102**:760–8
8. Wang X, O'Brien ME, Yu J, Xu C, Zhang Q, Lu S, Liang L, An X, McDyer JF, Mallampalli RK. Prolonged cold ischemia induces necroptotic cell death in ischemia–reperfusion injury and contributes to primary graft dysfunction after lung transplantation. *Am J Respir Cell Mol Biol* 2019;**61**:244–56
9. Krutsinger D, Reed RM, Blevins A, Puri V, De Oliveira NC, Zych B, Bolukbas S, Van Raemdonck D, Snell GI, Eberlein M. Lung transplantation from donation after cardiocirculatory death: a systematic review and meta-analysis. *J Heart Lung Transplant* 2015;**34**:675–84
10. Balibrea JM, Nunez-Pena JR, Garcia-Martin MC, Olmedilla Y, Martin-Antona E, Berthuin J, Rancan L, Vara E, Balibrea JL. The differential tissue expression of inflammatory, oxidative stress, and apoptosis markers in human uncontrolled non-heart-beating donors. *Transplantation* 2013;**95**:1346–53

11. Zhang J, Zhou H, Liu J, Meng C, Deng L, Li W. Protective effects of hydrogen inhalation during the warm ischemia phase against lung ischemia–reperfusion injury in rat donors after cardiac death. *Microvasc Res* 2019;**125**:103885
12. Floerchinger B, Oberhuber R, Tullius SG. Effects of brain death on organ quality and transplant outcome. *Transplant Rev* 2012;**26**:54–9
13. Zhou H, Fu Z, Wei Y, Liu J, Cui X, Yang W, Ding W, Pan P, Li W. Hydrogen inhalation decreases lung graft injury in brain-dead donor rats. *J Heart Lung Transplant* 2013;**32**:251–8
14. Kawamura T, Huang C-S, Peng X, Masutani K, Shigemura N, Billiar TR, Okumura M, Toyoda Y, Nakao A. The effect of donor treatment with hydrogen on lung allograft function in rats. *Surgery* 2011;**150**:2404–9
15. Tanaka Y, Shigemura N, Kawamura T, Noda K, Isse K, Stolz DB, Billiar TR, Toyoda Y, Bermudez CA, Lyons-Weiler J, Nakao A. Profiling molecular changes induced by hydrogen treatment of lung allografts prior to procurement. *Biochem Biophys Res Commun* 2012;**425**:873–9
16. Liu R, Fang X, Meng C, Xing J, Liu J, Yang W, Li W, Zhou H. Lung inflation with hydrogen during the cold ischemia phase decreases lung graft injury in rats. *Exp Biol Med* 2015;**240**:1214–22
17. Meng C, Ma L, Niu L, Cui X, Liu J, Kang J, Liu R, Xing J, Jiang C, Zhou H. Protection of donor lung inflation in the setting of cold ischemia against ischemia–reperfusion injury with carbon monoxide, hydrogen, or both in rats. *Life Sci* 2016;**151**:199–206
18. Zhang G, Li Z, Meng C, Kang J, Zhang M, Ma L, Zhou H. The anti-inflammatory effect of hydrogen on lung transplantation model of pulmonary microvascular endothelial cells during cold storage period. *Transplantation* 2018;**102**:1253–61
19. Kayawake H, Chen-Yoshikawa TF, Saito M, Yamagishi H, Yoshizawa A, Hirano S-I, Kurokawa R, Date H. Protective effects of a hydrogen-rich preservation solution in a canine lung transplantation model. *Ann Thorac Surg* 2021;**111**:246–52
20. Saito M, Chen-Yoshikawa TF, Takahashi M, Kayawake H, Yokoyama Y, Kurokawa R, Hirano SI, Date H. Protective effects of a hydrogen-rich solution during cold ischemia in rat lung transplantation. *J Thorac Cardiovasc Surg* 2020;**159**:2110–8
21. Takahashi M, Chen-Yoshikawa TF, Saito M, Tanaka S, Miyamoto E, Ohata K, Kondo T, Motoyama H, Hijiya K, Aoyama A, Date H. Immersing lungs in hydrogen-rich saline attenuates lung ischaemia–reperfusion injury. *Eur J Cardiothorac Surg* 2017;**51**:442–8
22. Noda K, Shigemura N, Tanaka Y, Bhama J, D'Cunha J, Kobayashi H, Luketich JD, Bermudez CA. Hydrogen preconditioning during *ex vivo* lung perfusion improves the quality of lung grafts in rats. *Transplantation* 2014;**98**:499–506
23. Haam S, Lee S, Paik HC, Park MS, Song JH, Lim BJ, Nakao A. The effects of hydrogen gas inhalation during *ex vivo* lung perfusion on donor lungs obtained after cardiac death. *Eur J Cardiothorac Surg* 2015;**48**:542–47
24. Haam S, Lee JG, Paik HC, Park MS, Lim BJ. Hydrogen gas inhalation during *ex vivo* lung perfusion of donor lungs recovered after cardiac death. *J Heart Lung Transplant* 2018;**37**:1271–8
25. Kawamura T, Huang CS, Tochigi N, Lee S, Shigemura N, Billiar TR, Okumura M, Nakao A, Toyoda Y. Inhaled hydrogen gas therapy for prevention of lung transplant-induced ischemia/reperfusion injury in rats. *Transplantation* 2010;**90**:1344–51
26. Li H, Zhou R, Liu J, Li Q, Zhang J, Mu J, Sun X. Hydrogen-rich saline attenuates lung ischemia–reperfusion injury in rabbits. *J Surg Res* 2012;**174**:e11–6
27. Ozeki N, Yamawaki-Ogata A, Narita Y, Mii S, Ushida K, Ito M, Hirano SI, Kurokawa R, Ohno K, Usui A. Hydrogen water alleviates obliterative airway disease in mice. *Gen Thorac Cardiovasc Surg* 2020;**68**:158–63
28. Noda K, Tanaka Y, Shigemura N, Kawamura T, Wang Y, Masutani K, Sun X, Toyoda Y, Bermudez CA, Nakao A. Hydrogen-supplemented drinking water protects cardiac allografts from inflammation-associated deterioration. *Transpl Int* 2012;**25**:1213–22
29. Sachdev S, Davies KJ. Production, detection, and adaptive responses to free radicals in exercise. *Free Radic Biol Med* 2008;**44**:215–23
30. Roy J, Galano JM, Durand T, Le Guennec JY, Lee JC. Physiological role of reactive oxygen species as promoters of natural defenses. *FASEB J* 2017;**31**:3729–45
31. Zhang J, Xue X, Han X, Li Y, Lu L, Li D, Fan S. Hydrogen-rich water ameliorates total body irradiation-induced hematopoietic stem cell injury by reducing hydroxyl radical. *Oxid Med Cell Longev* 2017;**2017**:8241678. doi: 10.11155/2017/8241678. Epub 2017 Jan 22. PMID: 28243358; PMCID: PMC5294227
32. Fang W, Tang L, Wang G, Lin J, Liao W, Pan W, Xu J. Molecular hydrogen protects human melanocytes from oxidative stress by activating Nrf2 signaling. *J Invest Dermatol* 2020;**140**:2230–41 e9
33. LeBaron TW, Kura B, Kalocayova B, Tribulova N, Slezak J. A new approach for the prevention and treatment of cardiovascular disorders. Molecular hydrogen significantly reduces the effects of oxidative stress. *Molecules* 2019;**24**:2076–102
34. Uto K, Sakamoto S, Que W, Shimata K, Hashimoto S, Sakisaka M, Narita Y, Yoshii D, Zhong L, Komohara Y, Li XK, Inomata Y, Hibi T. Hydrogen-rich solution attenuates cold ischemia–reperfusion injury in rat liver transplantation. *BMC Gastroenterol* 2019;**19**:25
35. Zhai Y, Zhou X, Dai Q, Fan Y, Huang X. Hydrogen-rich saline ameliorates lung injury associated with cecal ligation and puncture-induced sepsis in rats. *Exp Mol Pathol* 2015;**98**:268–76
36. Kawamura T, Wakabayashi N, Shigemura N, Huang CS, Masutani K, Tanaka Y, Noda K, Peng X, Takahashi T, Billiar TR, Okumura M, Toyoda Y, Kensler TW, Nakao A. Hydrogen gas reduces hyperoxic lung injury via the Nrf2 pathway *in vivo*. *Am J Physiol Lung Cell Mol Physiol* 2013;**304**:L646–56
37. Zhang Y, Liu Y, Zhang J. Saturated hydrogen saline attenuates endotoxin-induced lung dysfunction. *J Surg Res* 2015;**198**:41–9
38. Huang CS, Kawamura T, Peng X, Tochigi N, Shigemura N, Billiar TR, Nakao A, Toyoda Y. Hydrogen inhalation reduced epithelial apoptosis in ventilator-induced lung injury via a mechanism involving nuclear factor-kappa B activation. *Biochem Biophys Res Commun* 2011;**408**:253–8
39. Qiu X, Li H, Tang H, Jin Y, Li W, Sun Y, Feng P, Sun X, Xia Z. Hydrogen inhalation ameliorates lipopolysaccharide-induced acute lung injury in mice. *Int Immunopharmacol* 2011;**11**:2130–7
40. Saad A, Herrmann SMS, Eirin A, Ferguson CM, Glockner JF, Bjarnason H, McKusick MA, Misra S, Lerman LO, Textor SC. Phase 2a clinical trial of mitochondrial protection (elamipretide) during stent revascularization in patients with atherosclerotic renal artery stenosis. *Circ Cardiovasc Interv* 2017;**10**:e005487
41. Setsukinai K, Urano Y, Kakinuma K, Majima HJ, Nagano T. Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. *J Biol Chem* 2003;**278**:3170–5
42. Iuchi K, Nishimaki K, Kamimura N, Ohta S. Molecular hydrogen suppresses free-radical-induced cell death by mitigating fatty acid peroxidation a Nd mitochondrial dysfunction. *Can J Physiol Pharmacol* 2019;**97**:999–1005
43. Gvozdjáková A, Kucharská J, Kura B, Vančová O, Rausová Z, Sumbalová Z, Uličná O, Slezák J. A new insight into the molecular hydrogen effect on coenzyme Q and mitochondrial function of rats. *Can J Physiol Pharmacol* 2020;**98**:29–34
44. Sobue S, Inoue C, Hori F, Qiao S, Murate T, Ichihara M. Molecular hydrogen modulates gene expression via histone modification and induces the mitochondrial unfolded protein response. *Biochem Biophys Res Commun* 2017;**493**:318–24
45. Cui Y, Zhang H, Ji M, Jia M, Chen H, Yang J, Duan M. Hydrogen-rich saline attenuates neuronal ischemia–reperfusion injury by protecting mitochondrial fun ction in rats. *J Surg Res* 2014;**192**:564–72
46. Liu Q, Li BS, Song YJ, Hu MG, Lu JY, Gao A, Sun XJ, Guo XM, Liu R. Hydrogen-rich saline protects against mitochondrial dysfunction and apoptosis in mice with obstructive jaundice. *Mol Med Rep* 2016;**13**:3588–96
47. Zeeshan H, Lee G, Kim H-R, Chae H-J. Endoplasmic reticulum stress and associated ROS. *Int J Mol Sci* 2016;**17**:327
48. Gao Y, Yang H, Chi J, Xu Q, Zhao L, Yang W, Liu W, Yang W. Hydrogen gas attenuates myocardial ischemia reperfusion injury independent of postconditioning in rat s by attenuating endoplasmic reticulum stress-induced autophagy. *Cell Physiol Biochem* 2017;**43**:1503–14
49. Jiang S, Fan Q, Xu M, Cheng F, Li Z, Ding G, Geng L, Fu T. Hydrogen-rich saline protects intestinal epithelial tight junction barrier in rats

with intestinal ischemia–reperfusion injury by inhibiting endoplasmic reticulum stress-induced apoptosis pathway. *J Pediatr Surg* 2020;**55**:2811–9
50. Li H, Bai G, Ge Y, Zhang Q, Kong X, Meng W, Wang H. Hydrogen-rich saline protects against small-scale liver ischemia–reperfusion injury by inhibiting endoplasmic reticulum stress. *Life Sci* 2018;**194**:7–14
51. Lu Z, Lin Y, Peng B, Bao Z, Niu K, Gong J. Hydrogen-Rich saline ameliorates hepatic ischemia–reperfusion injury through regulation of endoplasmic reticulum stress and apoptosis. *Dig Dis Sci* 2017;**62**:3479–86
52. Bai X, Liu S, Yuan L, Xie Y, Li T, Wang L, Wang X, Zhang T, Qin S, Song G, Ge L, Wang Z. Hydrogen-rich saline mediates neuroprotection through the regulation of endoplasmic reticulum stress and autophagy under hypoxia-ischemia neonatal brain injury in mice. *Brain Res* 2016;**1646**:410–7
53. Sun Q, Han W, Hu H, Fan D, Li Y, Zhang Y, Lv Y, Li M, Pan S. Hydrogen alleviates hyperoxic acute lung injury related endoplasmic reticulum stress in rats through upregulation of SIRT1. *Free Radic Res* 2017;**51**:622–32
54. Chen HG, Han HZ, Li Y, Yu YH, Xie KL. Hydrogen alleviated organ injury and dysfunction in sepsis: the role of cross-talk between autophagy and endoplasmic reticulum stress: experimental research. *Int Immunopharmacol* 2020;**78**:106049
55. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol Life Sci CMLS*2016;**73**:3221–47
56. Diao M, Zhang S, Wu L, Huan L, Huang F, Cui Y, Lin Z. Hydrogen gas inhalation attenuates seawater instillation-induced acute lung injury via the Nrf2 pathway in rabbits. *Inflammation* 2016;**39**:2029–39
57. Yu Y, Yang Y, Yang M, Wang C, Xie K, Yu Y. Hydrogen gas reduces HMGB1 release in lung tissues of septic mice in an Nrf2/HO-1-dependent pathway. *Int Immunopharmacol* 2019;**69**:11–8
58. Li T, Deng S, Lei W, Li Z, Wu W, Zhang T, Dong Z. [Hydrogen water alleviates paraquat-induced lung fibroblast injury *in vitro* by enhancing Nrf2 expression]. *Nan Fang Yi Ke Da Xue Xue Bao* 2020;**40**:233–9
59. Lu Y, Li CF, Ping NN, Sun YY, Wang Z, Zhao GX, Yuan SH, Zibrila AI, Soong L, Liu JJ. Hydrogen-rich water alleviates cyclosporine A-induced nephrotoxicity via the Keap1/Nrf2 signaling pathway. *J Biochem Mol Toxicol* 2020;**34**:e22467
60. Kohama K, Yamashita H, Aoyama-Ishikawa M, Takahashi T, Billiar TR, Nishimura T, Kotani J, Nakao A. Hydrogen inhalation protects against acute lung injury induced by hemorrhagic shock and resuscitation. *Surgery* 2015;**158**:399–407
61. Xie K, Yu Y, Huang Y, Zheng L, Li J, Chen H, Han H, Hou L, Gong G, Wang G. Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis. *Shock* 2012;**37**:548–55
62. Tao B, Liu L, Wang N, Wang W, Jiang J, Zhang J. Effects of hydrogen-rich saline on aquaporin 1, 5 in septic rat lungs. *J Surg Res* 2016;**202**:291–8
63. Wittekindt OH, Dietl P. Aquaporins in the lung. *Pflugers Arch* 2019;**471**:519–32
64. Ge H, Zhu H, Xu N, Zhang D, Ou J, Wang G, Fang X, Zhou J, Song Y, Bai C. Increased lung ischemia–reperfusion injury in aquaporin 1-null mice is mediated via decreased hypoxia-inducible factor 2α stability. *Am J Respir Cell Mol Biol* 2016;**54**:882–91
65. Wu ZY, Yao Y, Hu R, Dai FF, Zhang H, Mao ZF. Cyclic adenosine monophosphate-protein kinase a signal pathway may be involved in pulmonary aquaporin-5 expression in ischemia/reperfusion rats following deep hypothermia cardiac arrest. *Genet Mol Res GMR* 2016;**15**:251
66. Hong-Min F, Chun-Rong H, Rui Z, Li-Na S, Ya-Jun W, Li L. CGRP 8-37 enhances lipopolysaccharide-induced acute lung injury and regulating aquaporin 1 and 5 expressions in rats. *J Physiol Biochem* 2016;**73**:381–6
67. Liu LD, Wu XY, Tao BD, Wang N, Zhang J. Protective effect and mechanism of hydrogen treatment on lung epithelial barrier dysfunction in rats with sepsis. *Genet Mol Res* 2016;**15**:9