

Emerging mechanisms and novel applications of hydrogen gas therapy

Nathanael Matei¹, Richard Camara¹, John H. Zhang^{1,2,3,*}

¹ Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA, USA

² Department of Anesthesiology, Loma Linda University, Loma Linda, CA, USA

³ Department of Neurosurgery, Loma Linda University, Loma Linda, CA, USA

*Correspondence to: John H. Zhang, MD, Johnzhang3910@yahoo.com.

orcid: 0000-0002-4319-4285 (John H. Zhang)

Abstract

Clinical and pre-clinical studies have reported a broad range of applications for hydrogen gas therapy. Classically, conventional antioxidant therapy is limited because it neutralizes both the detrimental and protective effects of reactive oxygen species. As a weak reducing agent, hydrogen gas avoids this paradox by reacting with strong oxidants while leaving other beneficial oxidants reactive. This review gathers a promising list of hydrogen gas applications that merit further mechanistic investigation and additional therapeutic trials. Reports support the ability of hydrogen gas to downregulate the expression of pro-inflammatory cytokines and pro-apoptotic factors. Mechanistically, hydrogen gas has been shown to downregulate miR-9 and miR-21, while upregulating miR-199 to reduce inflammatory injury. In angiogenic pathways, hydrogen's inhibition of cyclic guanosine monophosphate-degrading phosphodiesterase led to higher levels of cyclic guanosine monophosphate, activation of protein kinase, and angiogenesis; next, as hydrogen gas increased the levels of intracellular calcium, stimulated vascular endothelial growth factor increased nitric oxide production. In conjunction, hydrogen gas opened adenosine triphosphate-sensitive potassium channel channels, which activate downstream mitogen-activated protein kinase pathways. Growing molecular mechanisms have discovered a plethora of downstream targets for hydrogen gas therapy that include autophagy (*via* the adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin pathway), histone modification, mitochondrial unfolded protein response, acute oxidative stress after exercise, and oxidative stress secondary to aging. In conclusion, evolving research has discovered novel molecular connections that will continue to widen applications for hydrogen therapy.

Key words: hydrogen therapy; selective anti-oxidation, therapeutic applications; hydrogen pathway; anti-inflammation; anti-apoptosis; hydrogen gas; future directions

doi: 10.4103/2045-9912.239959

How to cite this article: Matei N, Camara R, Zhang JH. Emerging mechanisms and novel applications of hydrogen gas therapy. *Med Gas Res.* 2018;8(3):98-102.

Funding: This work was supported by the National Institutes of Health grant (NS081740 to JHZ).

INTRODUCTION

Clinical and pre-clinical studies have reported a broad range of applications for hydrogen gas therapy. Molecular hydrogen is the smallest gas molecule that can cross cellular membranes and diffuse throughout the body; however, it requires proper safety measures at concentrations above 4% because of its combustible properties.¹ The reaction constants of hydrogen with oxide radical ion ($\bullet\text{O}^-$) and hydroxyl radical ($\bullet\text{OH}$) in water is in the order of 10^{-7} M/s, compared to other molecules that are in the orders of 10^{-9} to 10^{-10} M/s; however, with high collision rates, hydrogen may overcome the low reaction rate constants and be a therapeutic antioxidant.^{1,2} Classically, conventional antioxidant therapy was limited because it neutralized both the detrimental and protective effects of reactive oxygen species (ROS): strongly oxidizing ROS, *e.g.*, $\bullet\text{OH}$, which damage tissue and advantageous species, superoxide and hydrogen peroxide, which enhance endogenous protective signal transduction pathways.³ As a weak reducing agent, hydrogen gas avoids this paradox by reacting with strong oxidants, *e.g.*, $\bullet\text{OH}$, while leaving other beneficial oxidants reactive.³

Hydrogen's unique reducing properties have been leveraged to treat a plethora of oxidative stress pathologies. Acute oxidative stress develops from vigorous exercise, inflammation,

ischemia and reperfusion (I/R) injury, surgical bleeding, and tissue transplantation.^{4,5} The first report of the therapeutic effects of hydrogen presented the regression of skin tumors in a mice model with squamous cell carcinoma.⁶ Studies have since broadened to include cardiovascular,⁷ nervous system,⁸ reproductive, metabolic,⁹ digestive,¹⁰ and respiratory diseases¹¹ (Figure 1).

While 12 L of endogenous hydrogen gas is produced daily by the intestinal flora, the mechanisms of low concentration hydrogen gas (1–4%) require further investigation,³ and although the mechanisms for hydrogen remain unclear, non-mechanistic studies have primarily focused on ischemic models. Molecular hydrogen behaves as an inert gas at body temperature and does not react with any biological compounds; therefore, with few side-effects and numerous publications on its biological and medical benefits, there is a promising potential for clinical use.¹² In this review, we hope to elucidate some of the emerging mechanisms and novel applications of hydrogen gas therapy.

Under aerobic conditions normal cellular functions produce ROS—*e.g.*, superoxide radical, $\bullet\text{OH}$ and hydrogen peroxide.¹³ During ischemia, mitochondrial damage occurs, causing electrons to leak from the Krebs cycle and the electron transport chain; these electrons produce superoxide anion



Applications for Hydrogen Therapy

- Inflammation
- Ischemia and reperfusion injury
- Surgical bleeding
- Tissue Transplantation
- Wrinkle formation
- Skin laxity
- Wound healing
- Inflammatory bowel disease
- Cardiovascular Diseases
- Nervous system diseases
- Reproductive system
- Metabolic Syndromes
- Digestive diseases
- Respiratory diseases
- Acute oxidative stress from vigorous exercise

Figure 1: Table of literature supported applications of hydrogen gas therapy.

radicals ($O_2^{\cdot-}$) which cause oxidative stress.¹⁴ Additionally, $O_2^{\cdot-}$ is produced by metabolic oxidases, NADPH and xanthine oxidases,¹³ converted into hydrogen peroxide (H_2O_2) by superoxide dismutase,¹⁵ and then detoxified into H_2O by either glutathione peroxidase or catalase.¹⁶ By way of the classically described Fenton Reaction, transitional ions, Fe^{3+} and Cu^{2+} , are reduced by copious amounts of $O_2^{\cdot-}$ and subsequently react with H_2O_2 to produce $\cdot OH$.¹⁷ Of importance, the strong oxidative and rapid diffusion properties of $\cdot OH$ lead to unpredictable non-specific reactions; in situ reactions produce oxidized bases, abasic sites, DNA-DNA intrastrand adducts, DNA strand breaks and DNA-protein cross-links.¹⁸ Finally, the acceptance of hydrogen gas as a therapy for oxidative stress is at least in part due to its preferential reduction of stronger oxidants, e.g., $\cdot OH$ and $ONOO^-$.²

ROUTES OF HYDROGEN ADMINISTRATION

Molecular hydrogen is usually administered by inhaling hydrogen-containing air (HCA) or by oral intake of hydrogen water (HW).¹⁹ Inhaled hydrogen gas is most suitable for defense against acute oxidative stress in emergency situations due to its rapid mechanism of action.¹⁶ In comparison, HCA showed superior distribution of hydrogen to both atrial and arterial blood, while HW increased hydrogen concentration by 10x in the atrial blood compared to that of the arterial blood.¹⁹ Additional studies found support for the hydrogen mediated reduction of $\cdot OH$ produced via the radiolysis or photolysis of water.²⁰ Similarly, Ohsawa et al.¹⁶ reported that hydrogen treatment (1–4% HCA) attenuated the deleterious damage of ROS, increasing neuronal survival and vitality, in both *in vitro* and *in vivo* ischemic models. Translationally, Ono et al.²¹

investigated the therapeutic effects of hydrogen gas (1–4%) in a randomized clinical study in patients with acute cerebral infarction and moderate-severity National Institute of Health Stroke Scale (NIHSS) scores 2–6. In this study, magnetic resonance imaging (MRI) findings suggested a reduced brain infarction accompanied with a quicker recovery time in the hydrogen group evaluated by NIHSS scores. No adverse effects were observed.²¹

Oxidative stress induced mitochondrial damage is one of the causes of Parkinson's disease (PD).²² Therapeutically, HW (0.08 ppm), made by dissolving electrolyzed hydrogen into pure water (H_2 bubbled water) or utilizing electrochemical reaction of magnesium with water (H_2/Mg water), a half-life of 2 hours, used for water consumption in animal models, significantly reduced dopaminergic neurons in substantia nigra after the injection with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the PD mouse model.²³ Additionally, more profound attenuation of oxidative stress and inflammatory responses was seen after treatment with HW in an Alzheimer's disease model in females compared to males²⁴; however, the sexual dimorphic differences require further investigation. Propitiously, HW can be administered frequently to overcome a short half-life and shows translational promise in preventative care and treatment for ROS associated pathologies.

In the neonatal hypoxia-ischemic rat model, hydrogen saline (HS) (5 mL/kg) reduced infarction ratio, increased neuronal survival, and reduced the number of apoptotic cells in part through suppression of caspase-3 activity.²⁵ Paralleling these findings, early brain injury in an endovascular puncture model of subarachnoid hemorrhage (SAH) was reduced by lowering neuronal apoptosis and associated protein markers (interleukin (IL)-6, Caspase-1, 3, IL-1 β , and tumor necrosis factor- α (TNF- α)) after HS treatment (5 mL/kg).²⁶ In a rat model of neuropathic pain induced by L5 spinal nerve ligation, intrathecal injection of HS (20 μ L of 0.6 mM HS) relieved allodynia and thermal hyperalgesia via reduction of spinal astrocytes and microglia, which are induced by an over abundance of $\cdot OH$ and $ONOO^-$.²⁷ Lastly, HS by intravitreal (20 μ L of 400 mL saline solution with hydrogen greater than 0.6 mM) and intraperitoneal (5 mL/kg) routes reduced retinal excitotoxic injury and promoted retinal recovery by the inhibition of glial cells, decreased expression of inducible NO synthase (iNOS) and 78 kDa glucose-regulated protein (GRP78), and increased clearance of glutamate.²⁸

Due to the hydrogen's ability to diffuse through the skin and into the blood stream, hydrogen bath research has grown and evolved in therapeutic applications. HW treatment can reverse the skin aging effects of ultraviolet radiation and ROS (wrinkle formation, laxity, leathery appearance, and impaired wound healing) by promoting type-1 collagen synthesis and significantly reducing human embryo fibroblast cell death after exposure to ultraviolet (UV $_A$).²⁹ Expanding the application for hydrogen gas therapy, excised cardiac grafts were submersed in a hydrogen-rich water bath (hydrogen concentration 0.5 mM) during cold preservation which attenuated cold ischemia/reperfusion injury in grafts from syngeneic older donors and allografts exposed to extended cold storage.³⁰ Moreover, retinal ischemia/reperfusion in-

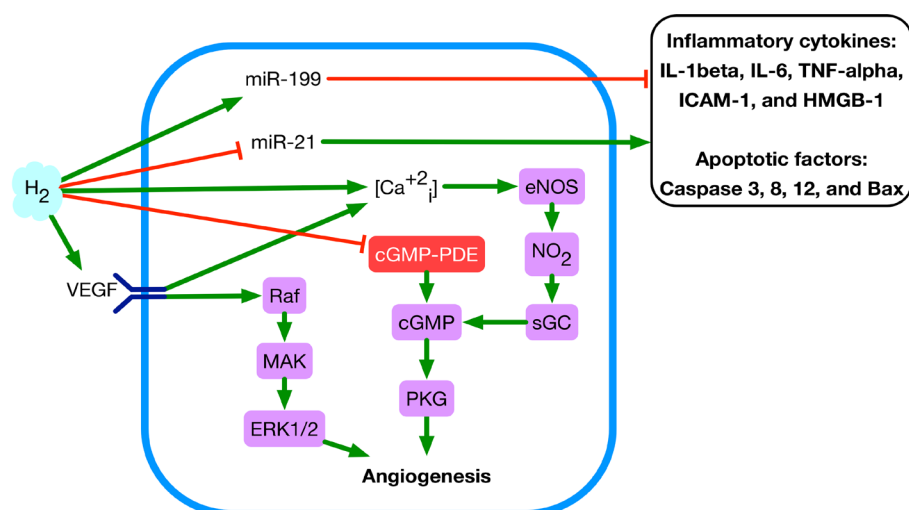


Figure 2: Proposed pathway for hydrogen (H₂) therapy and its downstream targets.

Note: VEGF: Vascular endothelial growth factor; MAK: serine/threonine-protein kinase; ERK1/2: extracellular signal-regulated kinase 1/2; cGMP: cyclic guanosine monophosphate; PDE: phosphodiesterases; PKG: protein kinase G; eNOS: endothelial nitric oxide synthase; NO₂: nitrogen dioxide; sGC: soluble guanylatecyclase; IL: interleukin; TNF: tumor necrosis factor; ICAM-1: intercellular cell adhesion molecule-1; HMGB-1: high-mobility group box protein 1.

jury in animal models by transient elevation of intraocular pressure and a mechanistic increase in ROS and subsequent apoptosis was reversed by the continuous delivery of hydrogen loaded eye drops to the ocular surface (4 mL/min, at saturated levels of hydrogen in solution) -- a significant reduction in retinal cell apoptosis.³¹ Clinically, hydrogen infused eye-drops used to target retinal ischemia may also treat ischemic optic neuropathies, obstructive arterial and venous retinopathies, carotid occlusive disorders, retinopathy of prematurity, chronic diabetic retinopathy and glaucoma.³² Current research of the direct incorporation of hydrogen has made significant therapeutic breakthroughs and, therefore, warrants further research to improve mechanistic understanding.

In inflammatory bowel disease, oxidation and inflammation activate macrophages and neutrophils which aggregate in the inflamed gut and elevate levels of ROS.³³ Mice with dextran sodium sulfate-induced colitis were given lactulose, fermented by bacteria in the colon, to produce high amounts of hydrogen, which significantly reduced colonic inflammation thus protecting the colonic mucosa.³³ Additionally, alpha-glucosidase inhibitor (acarbose) reduced postprandial hyperglycemia through the attenuation of disaccharide digestion in the small intestine and increased hydrogen fermentation of undigested carbohydrates by the resident enterobacterial flora, which was associated with a risk reduction of 25% for developing diabetes, 34% for developing *de novo* hypertension, and 49% for experiencing adverse cardiovascular events.^{34,35} The currently established endogenous potential of hydrogen gas, created for the neutralization of oxidative stress associated with inflammatory and cardiovascular pathologies, provides a foundational premise for future clinical trials.

BIOLOGICAL EFFECTS AND POTENTIAL MECHANISMS (SIGNALING PATHWAYS)

As previously mentioned, hydrogen gas penetrates biomembranes, even reaching the cell nuclei, and provides selective anti-oxidant, anti-inflammatory, and anti-apoptotic properties. In hypoxic environments, HT-22 cells upregulate the expression of miR-200 family which is reversed by the presence of hydrogen.³⁶ Further, retinal microglia cells activated with lipopolysaccharide (LPS) and then treated with hydrogen-saturated me-

dium showed significant downregulation of miR-9 and miR-21, while miR-199 upregulation reduced inflammatory injury.³⁷ Additionally, hydrogen gas downregulates the expression of pro-inflammatory cytokines, such as IL-1 β , IL-6, TNF- α , intercellular cell adhesion molecule-1 (ICAM-1), and high-mobility group box protein 1 (HMGB-1), and pro-apoptotic factors, such as caspase-3, caspase-12, caspase-8, and Bax.⁵

Furthermore, hydrogen gas has been shown to play an important role in the induction of angiogenesis which is necessary in neurogenesis, synaptogenesis, and neurosynaptic plasticity.³⁸ In a chicken chorioallantoic membrane, a simple model for investigating angiogenesis, hydrogen sulfide (H₂S) increased the length and complexity of the vascular network.³⁹ This mechanism was then studied in detail with endothelial cells *in vitro* to develop a working model for H₂S, and the following was established downstream: inhibition of cGMP phosphodiesterase led to higher levels of cGMP that activated protein kinase and promoted angiogenesis; next, as H₂S increased the levels of intracellular calcium, stimulated VEGF increased NO production.⁴⁰ In conjunction, H₂S opened KATP channels through direct interaction of Cys6 and Cys26, which activated downstream MAPK pathways.⁴¹ A working Hydrogen pathway model can be seen in Figure 2. Although enlightening, these mechanistic connections also expose insufficiencies that merit additional explorative research to generate additional therapeutic targets.

FUTURE DIRECTIONS

Addressing these caveats, hydrogen gas is an attractive therapy with a growing molecular mechanism justifying the treatment of additional pathologies. With developing potential, hydrogen therapy may play a significant role in the formation of pial collaterals that sprout through angiogenesis mechanisms and require paracrine VEGF-stimulation of Fik1-Notch signaling.⁴² These increased collaterals in the microcirculation are a primary clinical determinant of ischemic tissue damage after vascular obstruction.^{42,43} Lastly, recent studies have discovered a plethora of downstream targets of hydrogen gas therapy that include autophagy (via the adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) pathway),^{44,45} histone modification, mitochondrial

unfolded protein response,⁴⁶ acute oxidative stress after exercise,⁴⁷ and oxidative stress secondary to aging⁴⁸ (**Figure 3**). In conclusion, hydrogen gas has an expanding potential that is built upon an already established list of pathologies. Evolving research has discovered novel molecular connections that will continue to widen applications for hydrogen therapy. Our review suggests that a dearth of mechanistic knowledge and an already promising list of applications merit further mechanistic investigation and additional therapeutic trials for hydrogen gas.

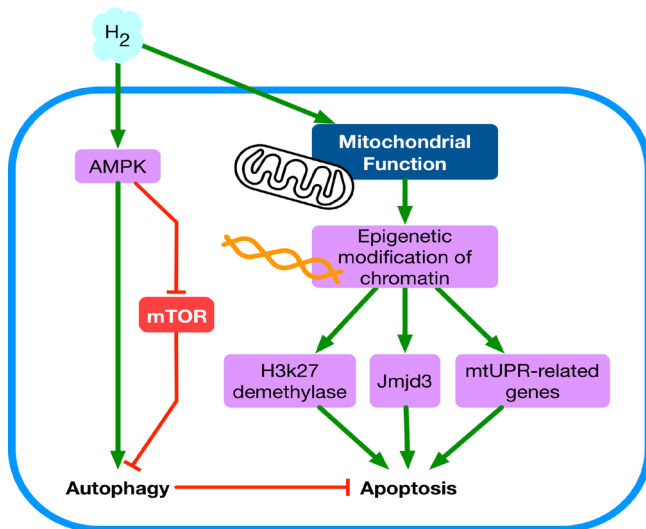


Figure 3: New targets discovered for hydrogen (H₂) therapy.

Note: The effects of hydrogen therapy are mediated in part through regulation of autophagy pathways and mitochondrial function. mtUPR: Unfolded protein response; AMPK: adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin; mTOR: mammalian target of rapamycin.

Author contributions

The idea and literature search were conducted by NM. Manuscript was drafted by NM. Critical revisions of the manuscript were made by all authors.

Conflicts of interest

The authors have no conflict of interest.

Financial support

This work was supported by the National Institutes of Health grant (NS081740 to JHZ).

Copyright license agreement

The Copyright License Agreement has been signed by all authors before publication.

Plagiarism check

Checked twice by iThenticate.

Peer review

Externally peer reviewed.

Open access statement

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

REFERENCES

- Ohno K, Ito M, Ichihara M, Ito M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. *Oxid Med Cell Longev*. 2012;2012:353152.
- Chuai Y, Gao F, Li B, et al. Hydrogen-rich saline attenuates radiation-induced male germ cell loss in mice through reducing hydroxyl radicals. *Biochem J*. 2012;442:49-56.
- Sano M, Suzuki M, Homma K, et al. Promising novel therapy with hydrogen gas for emergency and critical care medicine. *Acute Med Surg*. 2017;5:113-118.
- Vaziri ND, Rodríguez-Iturbe B. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. *Nat Clin Pract Nephrol*. 2006;2:582-593.
- Ge L, Yang M, Yang NN, Yin XX, Song WG. Molecular hydrogen: a preventive and therapeutic medical gas for various diseases. *Oncotarget*. 2017;8:102653-102673.
- Dole M, Wilson FR, Fife WP. Hyperbaric hydrogen therapy: a possible treatment for cancer. *Science*. 1975;190:152-154.
- Hayashida K, Sano M, Ohsawa I, et al. Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury. *Biochem Biophys Res Commun*. 2008;373:30-35.
- Nagata K, Nakashima-Kamimura N, Mikami T, Ohsawa I, Ohta S. Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent learning tasks during chronic physical restraint in mice. *Neuropsychopharmacology*. 2009;34:501-508.
- Furukawa S, Fujita T, Shimabukuro M, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004;114:1752-1761.
- Gharib B, Hanna S, Abdallahi OM, Lepidi H, Gardette B, De Reggi M. Anti-inflammatory properties of molecular hydrogen: investigation on parasite-induced liver inflammation. *C R Acad Sci III*. 2001;324:719-724.
- Zhou H, Fu Z, Wei Y, et al. Hydrogen inhalation decreases lung graft injury in brain-dead donor rats. *J Heart Lung Transplant*. 2013;32:251-258.
- Ohta S. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. *Pharmacol Ther*. 2014;144:1111.
- Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem*. 2001;11:173-186.
- Chinopoulos C, Adam-Vizi V. Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme. *FEBS J*. 2006;273:433-450.
- Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003;552:335-344.
- Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med*. 2007;13:688-694.
- Winterbourn CC. Toxicity of iron and hydrogen peroxide: the Fenton reaction. *Toxicol Lett*. 1995;82-83:969-974.
- Cadet J, Delatour T, Douki T, et al. Hydroxyl radicals and DNA base damage. *Mutat Res Mol Mech Mutagen*. 1999;424:9-21.
- Sobue S, Yamai K, Ito M, et al. Simultaneous oral and inhalational intake of molecular hydrogen additively suppresses signaling pathways in rodents. *Mol Cell Biochem*. 2015;403:231-241.
- Buxton GV, Greenstock CL, Helman WP, Ross AB. Critical Review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($\cdot\text{OH}/\cdot\text{O}^-$ in aqueous solution. *J Phys Chem Ref Data*. 1988;17:513-886.
- Ono H, Nishijima Y, Ohta S, et al. Hydrogen gas inhalation treatment in acute cerebral infarction: a randomized controlled clinical study on safety and neuroprotection. *J Stroke Cerebrovasc Dis*. 2017;26:2587-2594.
- Yamaguchi H, Kajitani K, Dan Y, et al. MTH1, an oxidized purine nucleoside triphosphatase, protects the dopamine neurons from oxidative damage in nucleic acids caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Cell Death Differ*. 2006;13:551-563.
- Fujita K, Seike T, Yutsudo N, et al. Hydrogen in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *PLoS One*. 2009;4:e7247.
- Hou C, Peng Y, Qin C, Fan F, Liu J, Long J. Hydrogen-rich water improves cognitive impairment gender-dependently in APP/PS1 mice without affecting A β clearance. *Free Radic Res*. 2018. doi: 10.1080/10715762.2018.1460749.
- Cai J, Kang Z, Liu K, et al. Neuroprotective effects of hydrogen saline in neonatal hypoxia-ischemia rat model. *Brain Res*. 2009;1256:129-137.



26. Shao A, Wu H, Hong Y, et al. Hydrogen-rich saline attenuated subarachnoid hemorrhage-induced early brain injury in rats by suppressing inflammatory response: possible involvement of NF- κ B pathway and NLRP3 inflammasome. *Mol Neurobiol*. 2016;53:3462-3476.
27. Ge Y, Wu F, Sun X, et al. Intrathecal infusion of hydrogen-rich normal saline attenuates neuropathic pain via inhibition of activation of spinal astrocytes and microglia in rats. *PLoS One*. 2014;9:e97436
28. Wei L, Ge L, Qin S, et al. Hydrogen-rich saline protects retina against glutamate-induced excitotoxic injury in guinea pig. *Exp Eye Res*. 2012;94:117-127.
29. Kato S, Saitoh Y, Iwai K, Miwa N. Hydrogen-rich electrolyzed warm water represses wrinkle formation against UVA ray together with type-I collagen production and oxidative-stress diminishment in fibroblasts and cell-injury prevention in keratinocytes. *J Photochem Photobiol B*. 2012;106:24-33.
30. Noda K, Shigemura N, Tanaka Y, et al. A novel method of preserving cardiac grafts using a hydrogen-rich water bath. *J Heart Lung Transplant*. 2013;32:241-550.
31. Oharazawa H, Igarashi T, Yokota T, et al. Protection of the retina by rapid diffusion of hydrogen: administration of hydrogen-loaded eye drops in retinal ischemia-reperfusion injury. *Invest Ophthalmol Vis Sci*. 2010;51:487-492.
32. Osborne NN, Casson RJ, Wood JPM, Chidlow G, Graham M, Melena J. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog Retin Eye Res*. 2004;23:91-147.
33. Chen X, Zhai X, Shi J, et al. Lactulose mediates suppression of dextran sodium sulfate-induced colon inflammation by increasing hydrogen production. *Dig Dis Sci*. 2013;58:1560-1568.
34. Chiasson JL, Josse RG, Gomis R, et al. Acarbose Treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM Trial. *JAMA*. 2003;290:486-494.
35. Suzuki Y, Sano M, Hayashida K, Ohsawa I, Ohta S, Fukuda K. Are the effects of α -glucosidase inhibitors on cardiovascular events related to elevated levels of hydrogen gas in the gastrointestinal tract? *FEBS Lett*. 2009;583:2157-2159.
36. Li H, Shen L, Ge J, Zhang R. The transfer of hydrogen from inert gas to therapeutic gas. *Med Gas Res*. 2017;7:265-272.
37. Liu GD, Zhang H, Wang L, Han Q, Zhou SF, Liu P. Molecular hydrogen regulates the expression of miR-9, miR-21 and miR-199 in LPS-activated retinal microglia cells. *Int J Ophthalmol*. 2013;6:280-285.
38. Ergul A, Alhusban A, Fagan SC. Angiogenesis: a harmonized target for recovery after stroke. *Stroke J Cereb Circ*. 2012;43:2270-2274.
39. Papapetropoulos A, Pyriochou A, Altaany Z, et al. Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc Natl Acad Sci U S A*. 2009;106:21972-21977.
40. Szabó C, Papapetropoulos A. Hydrogen sulphide and angiogenesis: mechanisms and applications. *Br J Pharmacol*. 2011;164:853-865.
41. Jiang B, Tang G, Cao K, Wu L, Wang R. Molecular mechanism for H₂S-induced activation of K(ATP) channels. *Antioxid Redox Signal*. 2010;12:1167-1178.
42. Lucitti JL, Mackey JK, Morrison J, Haigh JJ, Adams RH, Faber JE. Formation of the collateral circulation is regulated by vascular endothelial growth factor-A and a disintegrin and metalloprotease family members 10 and 17. *Circ Res*. 2012;111:1539-1550.
43. Meier P, Gloekler S, Zbinden R, et al. Beneficial effect of recruitable collaterals: a 10-year follow-up study in patients with stable coronary artery disease undergoing quantitative collateral measurements. *Circulation*. 2007;116:975-983.
44. Gao Y, Yang H, Chi J, et al. Hydrogen gas attenuates myocardial ischemia reperfusion injury independent of postconditioning in rats by attenuating endoplasmic reticulum stress-induced autophagy. *Cell Physiol Biochem*. 2017;43:1503-1514.
45. Yang F, Zhang L, Gao Z, et al. Exogenous H₂S protects against diabetic cardiomyopathy by activating autophagy via the AMPK/mTOR pathway. *Cell Physiol Biochem*. 2017;43:1168-1187.
46. 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;493:318-324.
47. Seifi B, Sajedizadeh A, Kadkhodae M, Ranjbaran M. Long-term exercise restores hydrogen sulfide in the kidney and contributes to exercise benefits in 5/6 nephrectomized rats. *Clin Exp Hypertens*. 2018. doi: 10.1080/10641963.2018.1445752
48. Hara F, Tatebe J, Watanabe I, Yamazaki J, Ikeda T, Morita T. Molecular hydrogen alleviates cellular senescence in endothelial cells. *Circ J Off J Jpn Circ Soc*. 2016;80:2037-2046.

Received: 2018-05-31

Accepted: 2018-08-03

C-Editor: Yang LJ, Zhao M; S-Editor: Yu J; L-Editor: Wang L; T-Editor: Jia Y