

# The potential utilizations of hydrogen as a promising therapeutic strategy against ocular diseases

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**Abstract:** Hydrogen<sup>1</sup>, one of the most well-known natural molecules, has been used in numerous medical applications owing to its ability to selectively neutralize cytotoxic reactive oxygen species and ameliorate hazardous inflammations. Hydrogen can exert protective effects on various reactive oxygen species-related diseases, including the transplantation-induced intestinal graft injury, chronic inflammation, ischemia–reperfusion injuries, and so on. Especially in the eye, hydrogen has been used to counteract multiple ocular pathologies in the ophthalmological models. Herein, the ophthalmological utilizations of hydrogen are systematically reviewed and the underlying mechanisms of hydrogen-induced beneficial effects are discussed. It is our hope that the protective effects of hydrogen, as evidenced by these pioneering studies, would enrich our pharmacological knowledge about this natural element and cast light into the discovery of a novel therapeutic strategy against ocular diseases.

**Keywords:** hydrogen, therapeutic strategy, ocular diseases

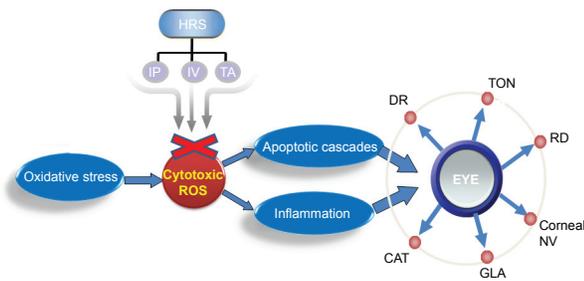
## Introduction

Reactive oxygen species (ROS) is a group of highly reactive molecules that are generated during energy-generating biochemical reactions and cellular functions.<sup>1–3</sup> Among them, physiological ROS are recognized as necessary signaling molecules in their own right. They are capable of oxidizing the phosphatases, kinases, as well as other vital proteins, and thereby efficiently modulate the metabolism and immune system.<sup>4</sup> Nevertheless, cytotoxic ROS subtypes, such as the hydroxyl radicals ( $\cdot\text{OH}$ ) and the peroxynitrite ( $\text{ONOO}^-$ ), can break down the mitochondrial membrane and promote the release of cytochrome-c, which further activates the downstream apoptotic cascade.<sup>5</sup> Thus timely elimination of these cytotoxic ROS is necessary without compromising other physiologically beneficial ROS, which are essentially correlated with the biological activities.

Generally, the enzymatic and nonenzymatic antioxidants build up an endogenous antioxidant defense to neutralize the cytotoxic ROS selectively. However, if the oxidative stress successively accumulates in the eyes and the protection from endogenous antioxidants is insufficient to neutralize the surplus ROS or maintain the cellular homeostasis, these excessive ROS would result in detrimental effects and ultimately cause cell death.<sup>6,7</sup> Hitherto, accumulating evidence suggests that the etiology of multiple ocular diseases, including cataract, glaucoma, age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy (DR), and multiple traumatic injuries,<sup>8</sup> are closely related to ROS. Therefore, it is necessary and reasonable to supply exogenous antioxidants to suppress the progression of ocular pathologies and alleviate the eyes from oxidative injury (Figure 1). The beneficial effects induced by

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**Figure 1** The basic principles of hydrogen-induced therapeutic effects against ophthalmological pathologies.

**Abbreviations:** CAT, cataract; Corneal NV, corneal neovascularization; DR, diabetic retinopathy; GLA, glaucoma; HRS, hydrogen-rich saline; IP, intraperitoneal; IV, intravitreal; RD, retinal degeneration; ROS, reactive oxygen species; TA, topical administration; TON, traumatic optic neuropathy.

the exogenous antioxidants to ameliorate ocular diseases are exciting.<sup>9,10</sup> However, several barriers can impede the therapeutic utilization of these exogenous scavengers, such as the low membrane permeability and the high toxic side effects. Furthermore, these disadvantages would constrain the administration of these therapies to a narrow window of therapeutic dosage.<sup>11</sup>

Hydrogen, a colorless, tasteless, and odorless diatomic gas, was initially recognized as a medical therapeutic substance in 1975.<sup>12</sup> Subsequently, Ohsawa et al<sup>13</sup> identified hydrogen-induced protective effects on a rat model of cerebral infarction in 2007 and highlighted the discovery of a gaseous radical scavenger. Thereafter, hydrogen-induced therapeutic effects on a wide range of diseases have been reported, including transplantation-induced intestinal graft injuries, cognitive deficits, inflammatory diseases, Parkinson's disease, metabolic syndromes, as well as ischemia–reperfusion (I/R) injury in the brain, liver, myocardium, intestines, and kidneys.<sup>14–22</sup>

Hydrogen can exert antioxidant and antiapoptotic activities via selectively neutralizing the cytotoxic ROS, such as the  $\cdot\text{OH}$  and  $\text{ONOO}^-$ . Meanwhile, the signaling ROS playing metabolic roles, such as the superoxide and hydrogen peroxide, are far less affected.<sup>13</sup> Unlike some antioxidant supplements with strong reductive reactivity, hydrogen neither disturbs the physiological oxidation reactions nor disrupts the essential defensive mechanisms. Recently, it has been verified that hydrogen can upregulate the expression levels of several endogenous antioxidant enzymes, including superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione transferase.<sup>23,24</sup> These advantages indicate that the application of hydrogen therapy might act as a safe and effective strategy against ROS-related disorders without giving rise to serious side effects.

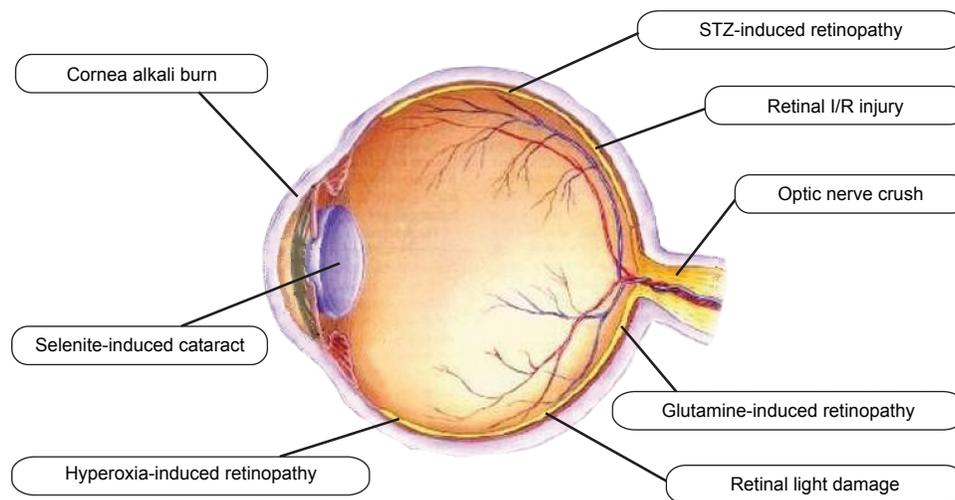
Hydrogen can penetrate the membranes and diffuse into organelles (eg, the mitochondria and nuclear DNA), and thereby get access to the intracellular source of cytotoxic ROS.<sup>13,24</sup> Based on the favorable distribution characteristic, hydrogen is highly effective for reducing the level of cytotoxic ROS, which impair the nuclear DNA and mitochondria. It has been found that inhalation of hydrogen is more effective than edaravone, an approved ROS scavenger in Japan, in alleviating the effects of ischemia-induced oxidative injury in neurons.<sup>25</sup> Furthermore, hydrogen is considered an anti-inflammatory because it significantly reduces the circulating levels of multiple proinflammatory cytokines, such as the chemokine (C–C motif), ligand 2 (CCL2), interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor- $\alpha$ .<sup>26–28</sup> Particularly, hydrogen can ameliorate the accumulation of activated microglia, which indicates inflammation and remodeling.<sup>29</sup>

Generally, hydrogen can be administered in a straightforward way by providing the gas for the subject to inhale using a ventilator circuit, facemask, or nasal cannula.<sup>30–32</sup> A subsequent study suggests that hydrogen can be dissolved in water up to 0.8 mM under atmospheric pressure at room temperature and its solubilized form, the hydrogen-rich saline (HRS), is advantageous since it is a safe, portable, and easily handled approach for delivery.<sup>24,33,34</sup> More importantly, a higher concentration of hydrogen can be dissolved and comprised in the HRS. Thus far, both *in vitro* and *in vivo* studies have verified that the antioxidant properties of HRS can decrease the incidence of ROS-related diseases. HRS can be administered orally, or in the form of peritoneal or intravenous injections.<sup>35–37</sup> Particularly, in the eye, it can be delivered by topical drops or intravitreal injections.<sup>38,39</sup>

The basic design and goal of potential clinical practice is to provide safe hydrogen delivery and precise efficiency analysis. The tissue compatibility of hydrogen is satisfactory because it is an endogenous substance, which is continuously produced in the human intestine.<sup>40</sup> From the aspect of safety, hydrogen is advantageous to many other antioxidants as it shows no cytotoxicity even at high concentrations. Inhalation of hydrogen has already been used in the prevention of decompression sickness in divers and provided good safety profiles.<sup>30</sup>

## The potential utilization of hydrogen against ocular diseases

Hydrogen can modulate several biological functions, and exhibits antioxidant and anti-inflammatory effects. The ability of hydrogen to neutralize free radicals, especially the hydroxyl radicals as well as other detrimental ROS, can



**Figure 2** The novel utilizations of hydrogen against multiple ophthalmological pathologies.  
**Abbreviations:** I/R, ischemia–reperfusion; STZ, streptozotocin.

be utilized to treat or prevent ocular disorders related to oxidative stress. In clinical practice, ophthalmologic utilization of hydrogen remains a rarely touched field. However, a series of pioneering experiments based on laboratory evidences have verified the valuable opportunities to develop hydrogen into a therapeutic tool against ocular diseases (Figure 2). Herein, we summarize the recent utilizations of hydrogen in the ophthalmological experiments and discuss its potential clinical feasibility (Table 1).

## The potential therapeutic effects against retinal degeneration

Retinal degeneration (RD) is a heterogeneous group of inherited retinal dystrophies that characterized by the progressive photoreceptor apoptosis.<sup>41–43</sup> Current therapeutic strategies against RD include the gene therapy, nutritional supplements, antiapoptotic therapy, retinal transplantation, retinal

prostheses, and stem cell therapy. However, the therapeutic effects of the aforementioned approaches remain unsatisfactory, owing to the complexity of etiology and the chronic cycle of pathological progression.<sup>44,45</sup> It has been shown that the ROS plays a pivotal role in caspase-independent photoreceptor apoptosis in RD.<sup>46</sup> This notion is further supported by the fact that multiple antioxidants have been successful in effectively suppressing photoreceptor degeneration in RD models.<sup>47–49</sup> Given the potent scavenging ability of hydrogen, it is reasonable to hypothesize that exogenous supplementation of hydrogen can ameliorate oxidative stress in RD and act as a therapeutic strategy to retard or prevent the photoreceptor degeneration.

Excessive exposure to light can induce the formation and accumulation of ROS in retinas and eventually results in the photoreceptors' apoptosis.<sup>43,49</sup> The pathologic mechanism of the light-induced RD is partly similar to that of RD.

**Table 1** Novel experimental investigations of hydrogen against the ocular pathologies (up to December 2015)

Animals	Disease models	Human diseases	HRS Delivery approach	Time and investigator
Rat	Retinal light damage	RD	IP injection	Tian et al <sup>51</sup>
Rat	Retinal light damage	RD	IP injection	Feng et al <sup>50</sup>
Rat	STZ induced retinopathy	DR	IP injection	Xiao et al <sup>56</sup>
Rat	STZ induced retinopathy	DR	IP injection	Feng et al <sup>57</sup>
Mouse	Hyperoxia induced retinopathy	DR	IP injection	Huang et al <sup>60</sup>
Rat	Optic nerve crush	TON	IP injection	Sun et al <sup>64</sup>
Rat	Selenite induce cataract	Cataract	IP injection	Yang et al <sup>23</sup>
Rat	Retinal I/R injury	Glaucoma	Topical drops	Oharazawa et al <sup>39</sup>
Rat	Retinal I/R injury	Glaucoma	IP injection	Liu et al <sup>77</sup>
Guinea pig	Glutamine induced retinopathy	Glaucoma	IP and IV injection	Wei et al <sup>38</sup>
Mouse	Cornea alkali-burn	Corneal neovascularization	Topical irrigation	Kubota et al <sup>71</sup>

**Abbreviations:** HRS, hydrogen rich saline; RD, retinal degeneration; DR, diabetic retinopathy; TON, traumatic optic neuropathy; IP, intraperitoneal injection; IV, intravitreal injection; STZ, streptozotocin.

Therefore, this highly reproducible model has been widely utilized in pathologic and therapeutic investigation of human RD. Hitherto, two independent studies have found that saturated hydrogen saline can protect the retina from light-induced damage by attenuating the oxidative stress.<sup>50,51</sup> It was shown that both retinal architecture and dark adapted electroretinogram (ERG) forms were relatively intact in hydrogen-treated rats compared with the vehicle-treated controls.<sup>51</sup> In greater detail, investigations using the electron microscope found that the hydrogen treatment could protect the cellular organelles of the photo receptors against the light induced injury. To some extent, these microstructure results verify that hydrogen can penetrate the membranes and then enter the nucleus and mitochondria. Moreover, the retinal malondialdehyde concentration, a presumptive marker of lipid peroxidation, was significantly reduced by hydrogen therapy, indicating that the protective effects of hydrogen could be ascribed to its antioxidant properties.<sup>50</sup> These encouraging findings based on the phototoxic models verify the efficacy of hydrogen to arrest photoreceptor degeneration and cast light on the discovery of a novel therapeutic to prevent the retinal damages in age-related macular degeneration or retinitis pigmentosa. From a long-term perspective, delaying the occurrence and progression of RD by clinical hydrogen therapy and establishing an efficient administrative protocol are the focus of future research.

### The potential therapeutic effects against DR

DR is the leading cause of blindness in the working force of developed countries and also acts as a significant cause of blindness in the elderly population.<sup>52</sup> The overall prevalence of retinopathy among patients with diabetes is around 26%. Both clinical and experimental studies have demonstrated that DR is closely related to oxidative stress: the ROS-induced biochemical changes can induce both structural and functional abnormalities in the microvasculature of diabetic retinas, resulting in the breakdown of blood–retinal barrier.<sup>53,54</sup> The blood–retinal barrier is critical for partitioning the neural elements of the retina from the circulation and protecting the retina from circulating inflammatory cells and cytotoxic products.<sup>55</sup> Consequently, ROS has been considered as a major contributor to the pathology of DR. A novel research sought to explore the effect of HRS on the streptozotocin-induced DR rat model. It was found that HRS can suppress caspase activity, ameliorate retinal apoptosis, and reduce vascular permeability.<sup>56</sup> Moreover, the HRS therapy can attenuate retinal parenchyma thickening. Another study

also adopted HRS to alleviate the streptozotocin-induced DR and found that the blood–retinal barrier in these treated rats was effectively preserved.<sup>57</sup>

The ROS could upregulate the expression levels of vascular endothelial growth factor (VEGF) and enhance VEGF receptor activity via complex signaling networks.<sup>58,59</sup> VEGF can give rise to choroidal neovascularization (CNV) in the DR and has been considered as a therapeutic target in clinical practice. A pioneering study based on the hyperoxia-induced mouse model found that HRS can counteract the CNV and reduce VEGF expression via inhibition of oxidative stress.<sup>60</sup> Therefore, the anti-VEGF property should be considered as another functional factor for hydrogen-induced protection. These preliminary studies indicate that hydrogen might act as a valid candidate in further clinical treatment of DR. Although the possible effect of hydrogen has not been clinically verified in DR patients hitherto, a randomized, double-blind, placebo-controlled, crossover study has already found that the supplementation of HRS improved lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance.<sup>61</sup> These hydrogen-induced effects might be potentially applied to the diabetic complications, especially to DR, in which metabolic disorder plays a pivotal role.

### The potential therapeutic effects against traumatic optic neuropathy

Traumatic optic neuropathy is one of the most devastating causes of permanent visual loss and complete blindness. After traumatic injury, such as the optic nerve crush, prominent signs of retinal ganglion cells (RGCs) apoptosis are found in the injured retinas.<sup>62</sup> Oxidative stress is now considered a pathologic factor that mediates the post-trauma process of RGCs apoptosis.<sup>63</sup> A pioneering study found that hydrogen can promote the survival of RGCs and enhance visual function recovery in an optic nerve crush rat model.<sup>64</sup> Moreover, the terminal deoxynucleotidyl transferase dUTP nick end labeling staining and malondialdehyde assay suggested that the neuroprotective effects were mediated by suppressing the ROS-induced apoptosis. These instrumental findings highlight the possibility of developing hydrogen into an effective tool against the traumatic optic neuropathy in clinical practice.

### The potential therapeutic effects against cataract

Cataract is the leading cause of blindness around the world and affects up to 80% of the human population over the age of

70 years.<sup>65</sup> Its prevalence is increasing in many countries due to the growth of aged population. Currently, the only effective treatment for cataract is surgical removal and replacement of the cataract with an artificial intraocular lens.<sup>66</sup> Nevertheless, an initial study suggested that hydrogen might act as an alternative therapy for the cataract.<sup>23</sup> It has been shown that hydrogen can retard cataract formation and restore the antioxidant capacity in a selenite cataract model by maintaining the activities of multiple enzymatic and nonenzymatic antioxidants. Malondialdehyde accumulation in the lenses of a selenite-treated rat was also efficiently suppressed, indicating that hydrogen possibly prevented the oxidation of lenticular membrane lipids. According to the classic theories, ROS is a crucial etiologic contributor to the initiation of cataract: ROS leads to a surge of detrimental biochemical reactions in the lens, including crosslinking and aggregation of lens proteins, peroxidation of membrane lipids, apoptosis of epithelial cells of the lens, and the eventual formation and progression of cataract.<sup>67</sup> In view of the potent ROS scavenging ability of hydrogen, it is reasonable to further explore its therapeutic effects for cataract pathology in clinical settings.

### The potential therapeutic effects against alkali injury of cornea

Accidental alkali burn is a terrible trauma to the eye that results in acute inflammation and secondary pathologic corneal neovascularization in the cornea. The balance between angiogenic and antiangiogenic factors determines the fate of corneal neovascularization as well as prognostic vision.<sup>68,69</sup> Thus far, existing therapies to prevent the onset of corneal neovascularization, such as the antiangiogenic drugs, argon laser photocoagulation, photodynamic therapy, and limbal stem cell transplantation et.al, are far from effective and efficient. ROS can activate the transcription nuclear factor kappa B (NF- $\kappa$ B), which then translocates to the nucleus to induce the expression of inflammatory cytokines such as VEGF, monocyte chemoattractant protein-1 (MCP-1), IL, and tumor necrosis factor- $\alpha$ .<sup>70</sup> These cytokines not only induce corneal neovascularization, but can also recruit inflammatory cells that further exacerbate inflammation. Therefore, ROS should be considered a key trigger for pathologic corneal neovascularization and inflammatory response in alkali-burned cornea. This hypothesis has been corroborated by an animal study: ROS can directly give rise to pathologic corneal neovascularization through the activation of the NF- $\kappa$ B pathway in the mouse cornea after an alkali-burn injury.<sup>71</sup> More importantly, the topical use of hydrogen-enriched solution can significantly reduce angiogenesis in these corneas. It was found that hydrogen water

irrigation significantly quenched NF- $\kappa$ B phosphorylation and abated the VEGF protein level. These encouraging findings suggest that the oxidative stress at the onset of corneal alkali injury can be an upstream target for therapeutic intervention. Hydrogen might be developed into a potent treatment against angiogenesis in alkali-burned corneas.

### The potential therapeutic effects against glaucoma

Transient elevation of intraocular pressure is known to induce retinal I/R injury and result in the necrosis and apoptosis of retinal neurons.<sup>72,73</sup> These pathological features closely resemble those of acute angle-closure glaucoma. The underlying mechanisms of I/R injury are closely related to the formation of ROS, which has been recognized as a contributor to the pathogenesis of glaucomatous neurodegeneration.<sup>74</sup> Encouragingly, it has been shown that the endogenous supplementation with antioxidant enzymes or ROS scavengers can retard or prevent I/R injuries in many mammal retinas.<sup>75,76</sup> In a recent study based on the rat model, continuous administration of hydrogen-loaded eye drops immediately increased the hydrogen concentration in the vitreous body and suppressed the I/R-induced oxidative stress, leading to a decrease in retinal neuron apoptosis.<sup>39</sup> Moreover, it has been verified that hydrogen treatment inhibits the activation of microglia, which could give rise to ongoing neurodegeneration in the injured retinas. Another study sought to explore the anti-apoptosis mechanism of HRS therapy found that HRS alleviated retinal I/R injury through the inhibition of poly ADP-ribose polymerase 1, a nuclear enzyme involved in the regulation of multiple pathophysiological cellular procedures, including DNA oxidation and caspase-3-mediated apoptosis.<sup>77</sup> These antiapoptotic and anti-inflammatory properties would inspire us to test the therapeutic effects of hydrogen against the glaucomatous pathologies in future clinical work.

Glutamate-induced excitotoxicity is another intraocular pressure-independent factor contributing to the apoptosis of RGCs in glaucoma.<sup>78</sup> Administration of HRS reduced glutamate excitotoxic injury and enhanced retinal recovery in guinea pigs.<sup>38</sup> These beneficial results could be ascribed to the suppression of the glial cells and the promotion of glutamate clearance. These underlying mechanisms will enrich our knowledge of hydrogen as a novel therapeutic element against glaucoma.

### Conclusion

Therapeutic medical gas can act as a reasonable approach for the treatment of oxidative stress-related disease. Hydrogen

is a promising gaseous agent that has come to the forefront of therapeutic research over the last few years. It can selectively reduce cytotoxic ROS and exert organ-protective effects through regulation of oxidative stress and inflammation. Hydrogen is so mild that it does not disturb metabolic oxidation–reduction reactions or disrupt the ROS involved in cell signaling. As highlighted earlier, the efficiency and safety of hydrogen in ameliorating ocular pathologies have been verified by a series of experiments. The pioneering attempts reviewed in the present work ignite the hope of discovering a novel, easy, and universal therapeutic tool against ocular diseases. Future hydrogen therapy should endeavor to establish well-defined administration guidelines and determine precise indications for clinical practice.

## Acknowledgment

This work is supported by the National Key Basic Research Program of China (No 2013CB967001).

## Disclosure

The authors report no conflicts of interest in this work.

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