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Use of Hydrogen as a Novel Therapeutic Strategy Against Photoreceptor Degeneration in Retinitis Pigmentosa Patients

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Statistical Analysis C
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Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies characterized by progressive photoreceptor apoptosis. Reactive oxygen species (ROS) have been recognized as critical initiators of the photoreceptor apoptosis in RP. Photoreceptor survival in RP mutants will not only require the inhibition of effectors of apoptotic machinery, but also the elimination of the initiating upstream signals, such as ROS. These cytotoxic ROS should be neutralized by the antioxidant defense system, otherwise they would interact with the macromolecules essential for photoreceptor survival. Hydrogen is a promising gaseous agent that has come to the forefront of therapeutic research over the last few years. It has been verified that hydrogen is capable of neutralizing the cytotoxic ROS selectively, rectifying abnormalities in the apoptotic cascades, and attenuating the related inflammatory response. Hydrogen is so mild that it does not disturb the metabolic oxidation-reduction reactions or disrupt the physiologic ROS involved in cell signaling. Based on these findings, we hypothesize that hydrogen might be an effective therapeutic agent to slow or prevent photoreceptor degeneration in RP retinas. It is a logical step to test hydrogen for therapeutic use in multiple RP animal models, and ultimately in human RP patients.

MeSH Keywords: **Hydrogen Peroxide • Retinitis Pigmentosa • Therapeutics**

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Background

Hydrogen, a colorless, tasteless, odorless, and highly flammable diatomic gas, was initially recognized as a medical therapeutic substance in 1975 [1]. Subsequently, Ohsawa and colleagues identified the astonishing protective effects of hydrogen in a rat model of cerebral infarction in 2007, and demonstrated the importance of discovering a gaseous radical scavenger [2]. Thereafter, the hydrogen-induced therapeutic effects on a wide range of diseases have been discovered, including transplantation-induced intestinal graft injuries; ischemia-reperfusion injury in the brain, liver, myocardium, intestine, and kidneys; cognitive deficits; inflammatory diseases related to oxidative stress; Parkinson's disease; and metabolic syndromes [3–13]. Hydrogen can exert therapeutic antioxidant and anti-apoptotic effects via selectively targeting the cytotoxic ROS, including OH⁻ and ONOO⁻. Signaling ROS playing metabolic roles, such as superoxide and hydrogen peroxide, are far less affected [2]. Unlike some antioxidant supplements with strong reductive reactivity, hydrogen does not disturb physiological oxidation reactions or disrupt essential defense mechanisms. These advantages could facilitate the use of hydrogen therapy as a safe and effective strategy against ROS-related disorders without giving rise to any serious adverse effects.

Hydrogen can penetrate membranes and diffuse into intracellular organelles (e.g., the mitochondria and nuclear organelles), and thereby access the intracellular source of cytotoxic ROS [2,14]. Based on these favorable distribution characteristics, hydrogen is highly effective in reducing cytotoxic ROS that impair the nuclear DNA and mitochondria. Furthermore, hydrogen is considered to be anti-inflammatory because it can significantly down-regulate the circulating levels of multiple proinflammatory cytokines. Particularly, hydrogen can ameliorate the accumulation of activated microglia, which indicates inflammation and remodeling [15]. Generally, hydrogen can be dissolved in water up to 0.8 mM under atmospheric pressure at room temperature and its solubilized form, hydrogen-rich saline (HRS), is advantageous because it is a safe, portable, and easily handled approach for delivery [16,17]. HRS could be administered orally, or by peritoneal or intravenous injections [18–20]. In the eye, it can be delivered by topical drops or intravitreal injections [21,22]. *In vitro* and *in vivo* studies have verified that the antioxidant properties of HRS can counteract oxidative stress. In terms of safety, abundant clinical and laboratory investigations have accumulated convincing profiles about the use of hydrogen in humans. Inhalation of hydrogen gas has already been used in the prevention of decompression sickness and in the treatment of diabetic patients [23]. Additionally, the tissue compatibility of hydrogen is quite satisfactory because it is an endogenous substance that is continuously produced in the human intestine [14].

Retinitis Pigmentosa (RP) and Oxidative Stress

RP is a heterogeneous group of inherited retinal dystrophies characterized by progressive photoreceptor degeneration [24]. The prevalence of RP is reported to be approximately 1 in 4000 individuals in Western countries, and more than 1.5 million RP patients have progressive visual deterioration due to this disorder [25]. The photoreceptor degeneration in the RP retina generally starts in the mid-periphery of the fundus and progresses towards the macula and fovea region, resulting in progressive narrowing of the field of vision. Experimental therapeutics against RP include gene rectification, neurotrophic factors supplements, anti-apoptotic therapy, retinal transplantation, nutritional dietary, visual prostheses, and stem cell therapy. However, due to the complexity of RP pathogenesis and the chronic cycle of RP pathology, the overall prognosis of RP remains dismal. Thus far, a nutritional approach with vitamin A at a dose of 15 000 IU per day is the only recommended clinical treatment for controlling RP, and the therapeutic effect is far less satisfactory [26].

Apoptosis is considered as the final common mechanism of the photoreceptor degeneration in multiple RP phenotypes [27]. The ongoing photoreceptor cell death in RP retinas runs in 2 parallel pathways: caspase-dependent and caspase-independent apoptosis [26]. Although different RP phenotypes have greatly differing heredity, these etiological mutations eventually converge to photoreceptor apoptosis. Therefore, the existence of a common cell death mechanism (e.g., apoptosis) triggered by different gene defects may provide a mutation-independent therapeutic target that can be generalized to all RP patients despite having different etiologies.

ROS has been recognized as a key contributor to the photoreceptor apoptosis of RP. ROS is a group of highly reactive, unstable molecules generated in a variety of energy-generating biochemical reactions and cellular functions [28–30]. These cytotoxic ROS subtypes, such as hydroxyl radicals (OH⁻) and peroxynitrite (ONOO⁻), can induce mitochondrial DNA stress and lipid oxidation, leading to mitochondrial membrane breakdown and the release of cytochrome-C, which further promotes the activation of downstream apoptotic cascades. In RP retinas, the cytotoxic ROS excessively elevate poly-ADP-ribose polymerase (PARP) activity and trigger photoreceptor apoptosis via interaction with transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) [31]. Furthermore, abundant ROS generated by the mitochondria could enhance cone apoptosis via the up-regulation of Bax and the down-modulation of Bcl-2 [32]. Additionally, a recent study has found that the peroxynitrite generated by ROS and nitric oxide (NO) exacerbates oxidative damage and contributes to photoreceptor death in RP. During amplification, NADPH oxidases (NOX)

serve as critical intermediaries [33]. Therefore, photoreceptor death in RP models will always be the combined results of multiple apoptotic pathways operating in parallel in complex networks [34]. The photoreceptor survival in RP mutants will not only require the inhibition of effectors of apoptotic machinery, but also entail the elimination of the initiating upstream signals, such as ROS. These cytotoxic ROS must be neutralized by the antioxidant defense system, otherwise they will interact with macromolecules, such as unsaturated lipids, proteins, deoxyribonucleic acid (DNA), and iron, which are critical for photoreceptor survival [35,36]. This notion is further supported by the abilities of multiple antioxidants to suppress photoreceptor apoptosis in RP animal models, and establish the role of ROS as a critical initiator of photoreceptor apoptosis.

Hypothesis

When oxidative stress accumulates in RP retinas and the protection by endogenous antioxidants is insufficient for maintaining retinal homeostasis or optimal visual function, it is necessary and reasonable to supplement exogenous antioxidants to protect the photoreceptors from oxidative injury and control the progression of RP. Hydrogen has been verified to be capable of selectively neutralizing cytotoxic ROS, rectifying the abnormalities in apoptotic cascades, and attenuating the inflammatory response. It might act as a natural agent to slow or prevent photoreceptor degeneration in RP retinas. Intravitreal injections of HRS, which may allow higher hydrogen concentrations and improve access to appropriate cellular compartments, would be an efficient method to deliver this therapeutic candidate to the targeted retinal tissue. Therefore, it is a logical next step to test HRS for therapeutic use in various RP animal models, and ultimately in human RP patients.

Evaluation of the Hypothesis

The enzymatic and nonenzymatic antioxidants in the retina build up an endogenous antioxidant defense to neutralize the cytotoxic ROS [36]. However, if oxidative stress accumulates in the pathologic retinas and the protection from endogenous antioxidants is insufficient to neutralize the excessive ROS or maintain cellular homeostasis, these surplus ROS can cause damage and ultimately lead to photoreceptor death [37,38]. Accumulating evidence suggests that the etiologies of several retinal diseases are correlated with ROS, including age-related macular degeneration, diabetic retinopathy, and multiple traumatic injuries [39–41]. Therefore, it is necessary and reasonable

to supply exogenous antioxidants to suppress the progression of ocular pathologies and protect the retina from oxidative insults. The beneficial effects of ROS scavengers in ameliorating photoreceptor degeneration of RP retinas have been reported in several studies [37,38]. Suppression of ROS-induced apoptosis by use of exogenous antioxidants not only protects individual cells, but also helps maintain the interconnected and inter-reliant web of the retina, which is pivotal for photoreceptor survival and visual signal transduction. Impediments to the therapeutic use of exogenous antioxidants include their low membrane permeability across the blood-retina barrier (BRB) and their highly toxic adverse effects, both of which would constrain the therapeutic dosage of these agents to a narrow window [42]. Because the BRB is generally compromised in RP eyes, most drugs are prevented from reaching effective doses in the retina. Theoretically, hydrogen could penetrate the membranes and diffuse into the organelles due to its lipid solubility and low molecular weight. However, the proven ability of hydrogen to cross the tight junctions of the BRB and thereby access the intra-retinal cytotoxic ROS should be determined by future research using hydrogen-sensitive probes.

Although several pioneering studies have validated hydrogen-induced beneficial effects in retinal pathology models, all of them were performed on experimental animals [39–41]. The mutation and the phenotype of the animal models cannot be assumed to truly represent the disease occurring in humans until the phenotypes are carefully examined using the same criteria. Accordingly, the laboratory effectiveness and related safety profiles must be viewed with caution. Hydrogen therapy must be administered at the right time, in the correct cellular compartment, and in appropriate concentrations to help RP patients. Currently, the ophthalmologic use of hydrogen is neglected in clinical practice. We cannot extrapolate a therapeutic dose of hydrogen for clinical use without good evidence of effectiveness and safety. Therefore, much more research is needed to translate this hypothesis into a useful therapeutic strategy for RP patients. The basic design of clinical practices is to provide safe hydrogen delivery and precise analysis of efficacy for RP patients. Further large-scale, randomized, controlled, double-masked clinical trials might demonstrate the feasibility of hydrogen treatment. Given the chronic progression of RP, establishing the long-term safety profiles and effective administrative protocol need to be the focus of hydrogen therapy research.

Conflicts of interest statement

All the authors declare that they have no conflicts of interest.

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