• Hypothesis •

A hypothesis for treating inflammation and oxidative stress with hydrogen sulfide during age-related macular degeneration

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Abstract

• Age-related macular degeneration (AMD) is a leading cause of blindness and is becoming a global crisis since affected people will increase to 288 million by 2040. Genetics, age, diabetes, gender, obesity, hypertension, race, hyperopia, iris-color, smoking, sun-light and pyroptosis have varying roles in AMD, but oxidative stress-induced inflammation remains a significant driver of pathobiology. Eye is a unique organ as it contains a remarkable oxygen-gradient that generates reactive oxygen species (ROS) which upregulates inflammatory pathways. ROS becomes a source of functional and morphological impairments in retinal pigment epithelium (RPE), endothelial cells and retinal ganglion cells. Reports demonstrated that hydrogen sulfide (H_2S) acts as a signaling molecule and that it may treat ailments. Therefore, we propose a novel hypothesis that H_2S may restore homeostasis in the eyes thereby reducing damage caused by oxidative injury and inflammation. Since H_2S has been shown to be a powerful antioxidant because of its free-radicals' inhibition properties in addition to its beneficial effects in age-related conditions, therefore, patients may benefit from H_2S salubrious effects not only by minimizing their oxidant and inflammatory injuries to retina but also by lowering retinal glutamate excitotoxicity.

• **KEYWORDS:** eye diseases; hydrogen sulfide treatment; inflammation; macula; oxidative stress; pyroptosis; retinal degeneration
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INTRODUCTION

Similar to many aging diseases such as neurodegeneration, diabetes, cancer and atherosclerosis, role of ocular inflammation mediated by the disruption of redox homeostasis has been studied extensively in age-related macular degeneration (AMD)[1]. Since retina is one of the highest oxygen consuming tissues in our body, it generates significant reactive oxygen species (ROS) moieties and related radical contents (Figure 1), which makes it vulnerable to oxidative injury over time[2-3]. A large amount of oxygen resides in the choroid and as oxygen tension falls across retinal pigment epithelium (RPE) and outer retina, it creates a vast oxygen gradient towards inner segments of the eyes' photoreceptor components. Also, photoreceptors in the retina contain relatively high levels of polyunsaturated fatty acids (PUFA) in comparison to other tissues. ROS-initiated lipid peroxidation reactions also generate reactive carbonyl compounds (RCC) from these biological lipids which further adds fuel to chronic neurodegenerative conditions such retinal degeneration[4]. Due to continuous accumulation of lipofuscin, which causes photo-oxidative damage (lipofuscin is a product of oxidation of lipids and lipoproteins containing photo-oxidative fluorophores such as green light-emitting retinol and retinyl esters), together with other photosensitizers. Abundant light exposure and a high metabolic demand make retina a prime location for the oxidative damage (Figure 1). The non-degradable

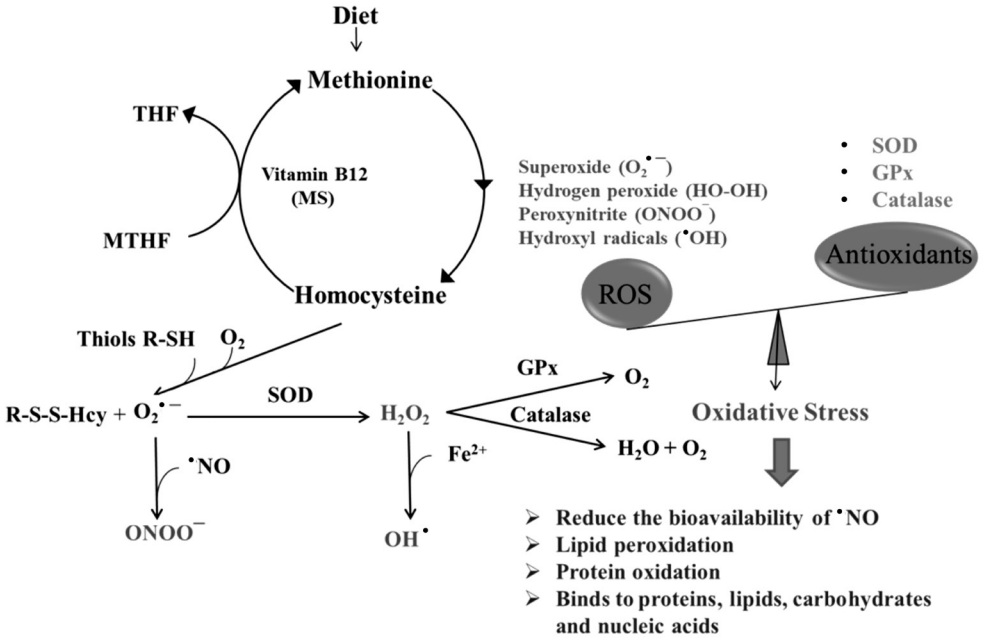

Figure 1 A simple schematic depicting harmful effects of hyperhomocysteinemia induced oxidative stress that causes inflammation because of redox disturbance The overall patho-physiological alterations are driven by oxidative stress, glutamate excitotoxicity and inflammation, and all together initiate and cause further worsening of the vision in AMD susceptible patient populations. GPx: Glutathione peroxidase; Hcy: Homocysteine; MS: Methionine synthase; THF: Tetrahydrofolate; MTHF: Methyltetrahydrofolate; SOD: Superoxide dismutase.

fluorophores which accumulate as lipofuscin inside RPE have been shown to cause RPE degeneration in AMD patients. For example, N-retinylidene-N-retinylethanolamine (A2E), the major component of lipofuscin, irreversibly damages RPE[5-10]. Moreover, during aging, oxidative damage also keeps increasing gradually because antioxidant capacity decreases concurrently in mammals. As a result, the inherent repair capacity of RPE cells becomes compromised[11-12]. The outcome is the retinal dysfunction which slowly leads to cells' loss and visual impairment because of the disruption in redox homeostasis. Different forms of RPE cell death are currently known to play important roles in AMD such as apoptosis, pyroptosis (cell death dependent on caspase-1) and necroptosis; the regulated necrosis dependent on receptor interacting protein kinase 3 and mixed lineage kinase domain-like but independent of caspases. All these retinal cell death pathways are important in AMD progression. In fact, ultrastructural investigations suggest that the predominant mechanisms of RPE cell death in AMD were mainly pyroptosis and necroptosis while apoptosis played only a minor role. Equally important though, some studies suggested that inflammasome activation can also alter the cell death pathway from apoptosis to pyroptosis as induced by photo-oxidation[7,13-14]. Such age-related changes are the hallmarks of AMD pathogenesis and along with genetic susceptibility and environmental factors they can further drive AMD pathology, eventually causing a full-blown AMD phenotype in patients[15]. World Health Organization (WHO) recently reported that retinal degenerative and vascular diseases have become the leading causes of blindness worldwide[16-17]. The fact that AMD is highly prevalent and can cause irreversible vision loss makes it an extremely important disease for ophthalmologists. Apart from AMD, oxidative injuries coupled with neurodegeneration are also involved in many other eye diseases as well, for which many studies have been published[18-20]. Aging, gene abnormalities and prominent metabolic stressors like hyperhomocysteinemia (HHcy) significantly increase oxidative stress, endoplasmic reticular (ER) stress and inflammation in the eyes of patients[21-27].

In past few years there has been a significant progress showing hydrogen sulfide (H_2S) as a novel molecule that has tremendous potential in the treatment of various systems' ailments[28-32]. In this manuscript, we discuss the potential beneficial effects of H_2S on retinal degenerative and vascular diseases (Figure 2). Retinal degenerative diseases, including retinitis pigmentosa, AMD and glaucomatous optic neuropathy, share the pathological basis of abnormal structure and function of retinal neurons, at all levels, and cause irreversible vision loss[33]. A recent study showed H_2S levels and expression of its endogenous enzymes cystathionine beta-synthase (CBS), cystathionine γ lyase (CSE) and 3-mercaptopyruvate sulfur transferase (3MST) in retinal tissues were significantly decreased along with the loss of retinal ganglion cells (RGCs) in a chronic ocular hypertension rat model. Furthermore, as briefly mentioned above, oxidative stress has been shown to play a harmful role in the development and progression of multiple neurodegenerative disorders including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and Huntington disease[34-35]. We and others have also shown that

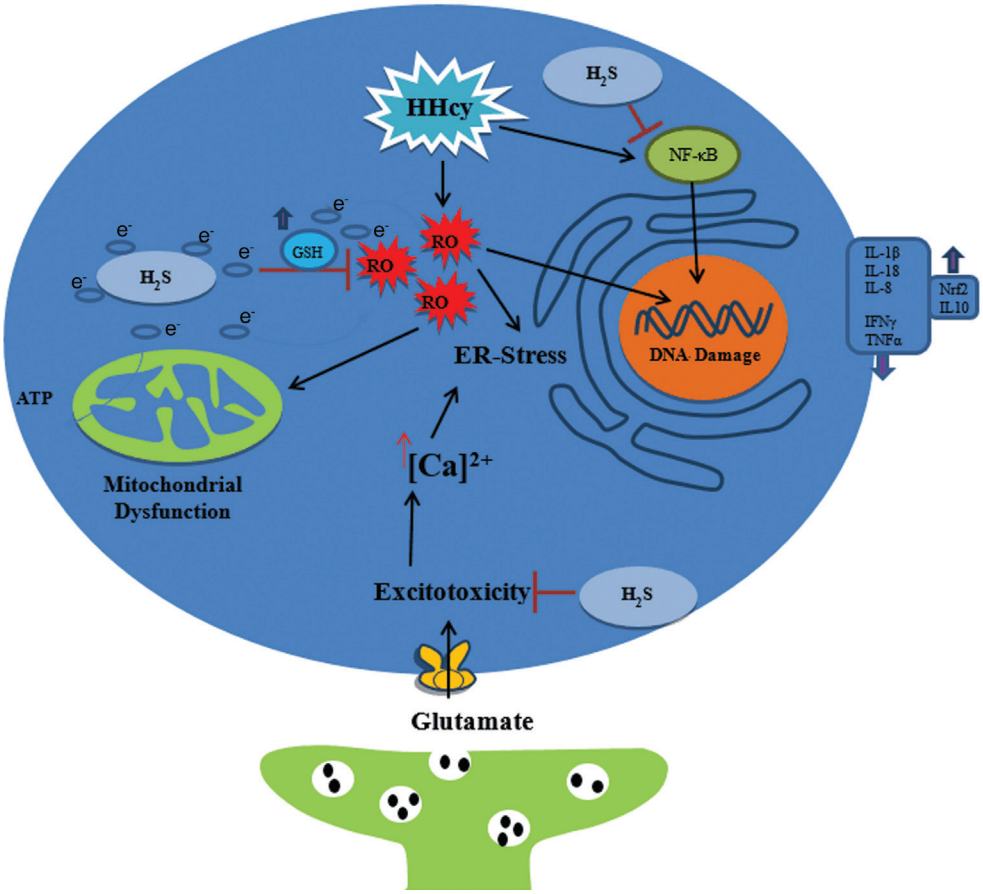

Figure 2 A cartoon highlighting the beneficial contributions of H_2S in reducing the deleterious effects as caused by HHcy mediated redox imbalance that leads to structural, physiological and functional changes in the retina Our proposed hypothesis explains that many harmful effects in the retina could be potentially alleviated by H_2S treatment, thus helping patients to protect or stabilize their vision during AMD who are relatively more prone to stress-related redox imbalance in their eyes. Ca^{2+}: Calcium; e^-: Electron; GSH: Glutathione; IFNγ: Interferon gamma; TNFα: Tumor necrosis factor alpha; Nrf2: Nuclear factor erythroid 2; IL-1β: Interleukin-1 beta; IL-18: Interleukin-18; IL-10: Interleukin-10; IL-8: Interleukin-8; NF-κB: Nuclear factor-kappa B.

H_2S does play a beneficial role in the physiology of nervous, cardiovascular, respiratory and gastrointestinal systems. It is an important and highly potent gasotransmitter produced by an enzymatic reaction in the brain and other tissues, and H_2S is involved physiologically in the process of neuro-regulation, vasodilatation and endocrine functions[35-38]. However, the actual role played by H_2S and its physiologically beneficial effects in the retina has yet to be fully realized. Nonetheless, some evidence does show that retina-derived H_2S plays a protective role in various aspects of its biology. Therefore, alleviation of oxidative injury and inflammation by H_2S is a novel approach that could help restore the dysfunctional redox homeostatic balance that is largely responsible for AMD (Figure 2).

HYPOTHESIS

It is well known that oxidative stress-mediated inflammation in susceptible eyes can initiate patho-physiological changes that can lead to macular degeneration (Figure 1). Several years ago we probed the molecular mechanisms that are triggered by HHcy inducing the oxidative damage-related stress responses[39]. As a result, now homocysteine-induced oxidative stress and production of ROS moieties are considered as the hallmark of antioxidant system failure[26,40-43]. As mentioned earlier, H_2S is an endogenous gaseous signaling molecule of significant physiological importance and is produced in various parts of the body such as the heart, blood, and central nervous system (CNS)[44]. H_2S is generated from L-cysteine by CBS, CSE, and/or 3MST. So far, only a few enzymatic pathways that regulate H_2S production have been studied: CBS, CSE, cysteine aminotransferase (CAT)/3MST and D-amino acid oxidase (DAO)/3MST. However, only the first three H_2S synthesis pathways have been reported to be involved in the retina[36,45-47]. CBS and CSE are expressed in the retinal tissue of salamander and those of CSE in the retinal tissue of mice[48]. Later, other groups detected the expression of H_2S-producing enzymes in almost every layer of the retina employing immunohistochemistry. These results also showed that 3MST and CAT were expressed in the inner plexiform layer, outer plexiform layer, inner nuclear layer, outer nuclear layer and outer segments of photoreceptors of the retina, with the absence

of CBS and CSE, which suggested that H_2S generation might be catalyzed mostly by the CAT/3MST pathway in the retina. Subsequently, there was further confirmation of CBS, CSE, and 3MST expression in retinal tissue by Western blot and immunohistochemistry analyses[49-51]. In CNS, H_2S has been reported to regulate synaptic activities as a neurotransmitter[52]. Ion channels and transporters were found to be involved in the regulatory effects of H_2S on CNS, as well[53-54]. The physiologic effects of H_2S in the retina along with its synthesis pathways and the fact that deficiency of CBS may lead to retinal degeneration and detachment indicate that H_2S does play an important role in the eye as a gaseous neuromodulator[55]. For example, by regulating Ca^{2+} influx, H_2S can protect retinal neurons against photo-toxicity (Figure 2). Excessive light exposure leads to photoreceptor degeneration and H_2S pre-conditioning can mediate the anti-apoptotic effects in retinal ischemia/reperfusion injury settings. Treatment with H_2S relieved the symptoms of diabetic retinopathy by suppressing harmful effects of oxidative stress along with reducing the debilitating effects of inflammation in the eyes. It appears that further studies would greatly improve our understanding of the detailed physiologic mechanisms responsible for retinal health and the potential of H_2S-centered therapy for the retinal diseases including AMD.

H_2S exhibited a prominent relaxation effect on the retinal arteries by acting on ion channels, meaning that it did play an important role in modulating the retinal physiology. For example, Voltage-gated Ca^{2+} channels: transient (T-type) and dihydropyridine-sensitive long-lasting (L-type) channels, have been reported to be expressed in Müller cells of the retina[56]. Several other ion channels also play roles in retinal degenerations, and these may interact with H_2S. Disturbances in calcium transport system exist in retinal Müller cells as well as in the RPE[57-58]. Furthermore, sodium and chloride channels have important effects on various physiologic processes in the retina[59-62]. Therefore, more experimental studies are warranted to explore relationships between retina-derived H_2S and the ion channels that are closely linked together in the rapid excitatory synaptic transmission processes of CNS. Also, the glutamate aspartate transporter in retinal Müller cells is involved in maintaining the levels of glutathione (GSH)[63]. The oxidative stress mediated inflammatory process in the eyes of susceptible hosts that can lead to the beginning of subtle pathological changes are known to trigger the degenerative and inflammatory cascades in the retina[26].

Age-related dysregulation of immune response in the retina can contribute to disease pathogenesis[64-65]. As microglia are the primary resident immune cell in the retina, and are long-lived cells that persist across long periods of chronological time senescent changes occurring within aging microglia may be one cause of immune response “failure”, conferring upon the retina an age-dependent vulnerability to disease[66-67]. Glutamate can also activate microglia and enhance cytokine-induced neurodegeneration[68]. Therefore, we believe that this transporter which regulates neurotransmission in the retina is related to glutamate excitotoxicity, might be a potential target of H_2S treatment. Even under such conditions, the treatment with H_2S may also offer clinical benefits for alleviating excitotoxicity, and related ER stress conditions arising from the disturbed glutamatergic system operated cascade of events that invariably lead to microglial activation and inflammation (Figure 2). During the oxidative stress mediated glutamate excitotoxicity the extracellular concentrations of glutamate are increased and results in import of cystine in exchange for glutamate by the cystine/glutamate antiporter. Because cystine is reduced to cysteine in cells for the synthesis of GSH, a decrease in the cystine import results in the decreased synthesis of GSH. The enhanced glutamate level is also involved in microglia activation. Briefly, calcium dysregulation, ER stress and mitochondrial impairment and microglia activation are the major components of glutamate excitotoxicity. It appears that H_2S reinstates the cystine import suppressed by glutamate. In a nutshell, it appears that H_2S treatment might lower glutamate excitotoxicity, ER stress and microglial activation which are all linked to oxidative stress thus offering a potential interventional strategy for many ocular diseases including AMD.

CONCLUSION

The existence of endogenous H_2S synthesis pathways in the mammalian retina and the physiological roles played by this important gasotransmitter makes it an ideal candidate to further explore its use in the treatment and prevention of chronic retinal diseases such as AMD. For example, by regulating Ca^{2+} influx, H_2S can protect retinal neurons against light-induced degenerative events. Thus, H_2S-based preconditioning can be employed to avoid development of chronic injury from oxidative stress or inflammation in AMD[69-70]. AMD is one of the leading causes of vision impairment worldwide, and thus new approaches are urgently needed to develop effective treatment and preventive options. Several treatments have been developed, such as anti-oxidant supplements to slow the progression of dry form of AMD, and photodynamic therapy and anti-VEGF agents to treat the wet form of AMD; a more advanced form of AMD characterized by choroidal neovascularization under the macula. However, there remains neither a definitive preventive measure nor a cure for this dreaded disease[71-72]. Interestingly, HHcy-mediated oxidative stress has also been implicated in the pathogenesis of several vascular diseases[39]. Because of the inherent properties of H_2S, it can easily penetrate plasma membranes, thus inducing a wide spectrum of signaling cascades in target cells (Figure 2). Studies employing cellular and animal models have

suggested a number of mechanisms to explain the protection associated with H_2S including reduction of mitochondrial damage[73-75], scavenging oxygen derived free radicals, reducing inflammation[76-81] apoptosis, and increasing vasodilation and neuroprotection[82-84]. H_2S also increases the production of intracellular GSH, a major intracellular antioxidant which promotes vascular and neuronal protection[85-88]. As we know, unlike the CNS or cardiovascular system, a unique characteristic of the retina is its direct connection to the vitreous body, which is a perfect match to gaseous treatment modalities. To summarize, H_2S has already proved beneficial as a neuromodulator agent in the eye. It is a suitable molecule to test further for its beneficial effects against oxidative stress, be it induced by HHcy, glutamate excitotoxicity, or ER stress. This novel hypothesis-centered strategy might curtail AMD progression by treating the oxidation-induced inflammation underlying AMD and other neurodegenerative diseases (Figures 1, 2).

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