Hydrogen Sulfide Attenuates High Glucose-Induced Human Retinal Pigment Epithelial Cell Inflammation by Inhibiting ROS Formation and NLRP3 Inflammasome Activation

Peng Wang, Fei Chen, Wenyan Wang, and Xue-Dong Zhang

The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, China

Correspondence should be addressed to Xue-Dong Zhang; zxued@sina.com

Received 16 December 2018; Revised 24 March 2019; Accepted 31 March 2019; Published 24 April 2019

1. Introduction

Diabetic retinopathy (DR) is a common complication of diabetes and is also the leading cause of visual impairment and blindness [1]. It has been well recognized that hyperglycemia plays a pivotal role in the pathogenesis of DR. The chronic hyperglycemic environment damages not only the retinal vasculature but also other types of cells in the retina, such as retinal pigment epithelial (RPE) cells, which are a crucial component of the blood-retinal barrier and a key element in maintaining the proper function of the visual system [1, 2].

DR has been caused by a combination of multiple factors, although the etiology and progression of DR remain poorly understood. Several mechanisms have been found to be closely associated with DR. These include the activated polyol pathway and protein kinase C (PKC), increased expression of various growth factors, vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1), and hemodynamic changes and the formation of advanced glycation end products (AGEs) [3]. Furthermore, it has also been well documented that increased oxidative stress, the activation of the renin-angiotensin-aldosterone system (RAAS), and subclinical inflammation and capillary occlusion also play important roles in the pathogenesis of DR [3, 4].

Recent studies have indicated that the pathogenic effect triggered by oxidative stress was induced via the promotion of inflammatory response and apoptosis through the activation of the downstream signaling pathway [5–7]. Furthermore, moderate levels of reactive oxidative species (ROS) play an important role in the immune response to foreign pathogens, while high levels of ROS often lead to pathogenesis and disease.
In diabetes mellitus, oxidative stress can be activated through the polyol pathway, PKC, AGEs, amidohexose, and other metabolic pathways [8, 9], and such extensive levels of ROS could subsequently lead to neovascularization and the activation of inflammatory cytokines [10], which in turn accelerates DR progression.

The NLRP3 inflammasome, which is a complex formed by NLRP3, ASC, and caspase-1, is a key element in inflammatory immune response via caspase-1 activation and proinflammatory cytokine IL-1β and IL-18 secretion after activation [11]. Recent studies have indicated that mitochondrial ROS generation is closely linked with the activation of NLRP3, suggesting that the NLRP3 inflammasome is a critical sensor of mitochondrial dysfunction, and that this might well explain why many metabolic disorders are associated with mitochondrial damage [12]. NLRP3 has also been reported to play an important role in diabetes and other metabolic diseases [13]. A recent study revealed that high glucose could stimulate ROS formation and NLRP3 inflammasome activation and eventually increase cell apoptosis and retinal vascular permeability in human retinal microvascular endothelial cells (HRMECs) and the retina of diabetic rats [14]. In addition, blocking NLRP3 inflammasome activation by siRNA silencing could attenuate these effects [14].

Hydrogen sulfide (H₂S) is known to be synthesized intracellularly from cysteine by cystathionine gamma-lyase (CSE) and obtained from other naturally occurring enzymes. Furthermore, H₂S has been reported to play an important role in the pathophysiology of the nervous system, circulatory system, immune system, and endocrine system [15]. Growing evidence suggests that H₂S has a protective role against various inflammatory-stimulated injuries in tissues, such as those from the heart, liver, and kidneys [16-18]. Furthermore, H₂S mainly works as an antioxidant [19]. On the one hand, it has been reported that the addition of H₂S could increase the reduced form of intracellular glutathione (GSH) in a monocyte cell model [20]. On the other hand, H₂S has been reported to directly scavenge superoxide anions, hydrogen peroxide (H₂O₂), and peroxynitrite [21]. Furthermore, H₂S and its endogenous enzymes have been considered to play a pivotal role in the pathogenesis of diabetes and its complications, since studies have confirmed that H₂S levels decrease in diabetic patients and diabetic rats [22, 23] and, in particular, tissues with diabetic endothelial dysfunction, nephropathy, and cardiomyopathy [24, 25]. Moreover, it has been shown that retinal tissue can also produce H₂S, which might be involved in the pathogenesis of retinal degeneration and retinal ischemia-reperfusion injury [26]. Based on these findings, the present study intended to investigate whether exogenous H₂S could protect human retinal pigment epithelial (ARPE-19) cells against high glucose-induced damage and its role in anti-inflammation, especially the NLRP3 inflammasome.

2. Materials and Methods

2.1. Materials. Sodium hydrosulfide (NaHS) was purchased from Chuandong Chemical Group Co. Ltd. (Chongqing, China). N-Acetyl-L-cysteine (NAC) and 2,7-dichlorofluorescein diacetate (DCFH-DA) were manufactured by Sigma-Aldrich (St. Louis, MO, USA). Cell Counter Kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). Dulbecco’s modified Eagle’s medium F12 (DMEM/F12) and fetal bovine serum (FBS) were purchased from Gibco-BRL (Carlsbad, CA). TRIzol RNA isolation reagent was purchased from Invitrogen Life Technologies (New York, New York, USA). The first strand cDNA synthesis kit and SYBR green reagents were purchased from Takara (Dalian, China). siRNA of NLRP3 was obtained from Hanbio Biotechnology Co. Ltd. (Shanghai, China).

2.2. Cell Culture. Human retinal pigment epithelium cell line ARPE-19 was obtained from the American Type Culture Collection (ATCC). Cells were cultured in (1:1) mixed Dulbecco’s modified Eagle’s medium: nutrient mixture F12 (DMEM/F12), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) and antibiotics (100 mg/mL of streptomycin and 100 U/mL of penicillin). Then, cells were cultured at 37°C in a humidified chamber with 5% CO₂ and passed every 5-7 days. Trypan-EDTA solution (diluted from 1:3 to 1:4) was used to dissociate cells from the culture flasks (Corning, Lowell, MA, USA) after cells reached full confluency. Six testing cell culture groups were established: (1) LG: low-glucose group (5.5 mM of glucose), (2) HG: high-glucose group (25 mM of glucose), (3) NAC: high-glucose+NAC group (2.5 mM of NAC in 25 mM of glucose medium), (4) NLRP3 siRNA: high-glucose+NLRP3 siRNA group (cells were transfected with NLRP3 siRNA), (5) control siRNA: high-glucose+scrabble siRNA group (cells were transfected with scramble siRNA), and (6) H₂S: high-glucose+H₂S group (200 μM of NaHS in 25 mM of glucose medium). Cells in each group were induced for 48 hours before further experimental tests.

2.3. Cell Viability Assay. ARPE-19 cells were cultured in 96-well plates with or without the various treatments. Then, a 10 μL CCK-8 solution (1:10 dilution) was added to each well and incubated for an additional two hours. The absorbance at 450 nm was recorded using a microplate reader, and cell viability was calculated according to the manufacturer’s instructions.

2.4. ELISA. IL-18 and IL-1β levels from the supernatant of homogenized cultured cells were measured by ELISA. An IL-1β ELISA kit (eBioscience; Ref: BMS630, Lot: 87225015) and an IL-18 ELISA kit (Novex; Ref: KRC2341, Lot: 130401/A) were used for the analysis. Each sample was tested at least three times.

2.5. Measurement of Intracellular ROS. After various treatments, 10 μM of DCFH-DA was added to each well and incubated at 37°C for 30 minutes. Then, cells were washed with PBS for three times. Next, cells were resuspended in PBS at 1 × 10⁶ cells/mL, and the fluorescence of the oxidized dichlorofluorescein (DCF) product was measured via fluorescence-activated cell sorting (FACS) flow cytometry at an excitation of 488 nm and an emission of 525 nm. Untreated cells served as controls. These results
were expressed as the fluorescence intensity of DCF relative to control.

2.6. Real-Time Polymerase Chain Reaction (PCR). Total RNA was extracted and isolated from ARPE-19 cells using the TRizol reagent (Life Technologies, New York, USA), according to the manufacturer’s instructions. Quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA, USA).

The primers (Table 1) were designed and synthesized by Sangon Biotech (Shanghai, China). Real-time PCR was performed using the SYBR Green master mix, according to the standard thermocycler conditions. The target gene expression was quantified relative to the housekeeping gene β-actin via the optimized comparative 2^(-ΔΔCT) method, as reported by a previous study [27].

2.7. Antioxidant Intervention. For the antioxidant-related test, NAC at a concentration of 2.5 mM was used to treat ARPE-19 cells cultured under low- and high-glucose conditions for 48 hours. Then, cells were collected and subjected to RT-PCR and ELISA.

2.8. Gene Silencing Assay. NLRP3 siRNA with its sequence of 5'-CAGGTGTITCGAATCCACTGTGAT-3' and scrabble siRNA with its sequence of 5'-CATGGATTGGTGAACAGCCACCTCA-3' were obtained from Hanbio Biotechnology Co. Ltd. (Shanghai, China). The siRNA was delivered using Lipofectamine 2000 (Life Technologies, California, USA), according to the manufacturer’s instruction. After siRNA transfection, cells were cultured for another 48 hours, and the supernatant was collected for ELISA.

2.9. Statistical Analysis. All data were expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used to determine the significance between groups using the SPSS software (version 17.0, Chicago, IL, USA). A P value < 0.05 was considered statistically significant.

3. Results

3.1. High Glucose Decreases ARPE-19 Cell Viability and Induces Inflammatory Cytokine Expression. To investigate the effect of the cell viability of RPE cells, human retinal pigment epithelial (ARPE-19) cells were cultured in medium with low glucose (5.5 mM) and high glucose (25 mM) for 48 hours, respectively. Cell viability was determined by the CCK-8 assay. As shown in Figure 1(a), a significantly reduced cell viability was observed in the 25 mM glucose culture condition, when compared with the 5.5 mM glucose medium (P < 0.01), which is consistent with a previous study conducted by the investigators [28]. But as shown in Figure 1(a), the NaHS and NAC could significantly attenuate high glucose-induced reduction of cell viability.

To further understand the cell responses under high-glucose stimulation, the intracellular production of two inflammatory cytokines IL-18 and IL-1β was measured by ELISA. As illustrated in Figures 1(b) and 1(c) and compared with the low-glucose culture condition, high glucose triggered an approximately 65% and 72% increase in IL-18 and IL-1β production in ARPE-19 cells, respectively (P < 0.01). Furthermore, the mRNA levels of IL-18 and IL-1β in ARPE-19 cells were also measured under high- vs. low-glucose culture conditions. As shown in Figures 1(d) and 1(e), similar results were found in mRNA expression relative to protein production.

3.2. High Glucose Increases Intracellular ROS Formation and Activates the NLRP3 Inflammasome in ARPE-19 Cells. The increase in proinflammatory cytokine formation by high glucose inspired the investigators to determine the ROS production and inflammasome activation in RPE cells. To determine the ROS, cells were labeled with a fluorescence marker DCFH-DA, and the redox sensor and DCF fluorescence would form once DCFH-DA becomes intracellularly oxidized. Then, cells were quantitatively monitored by FACS flow cytometry. As indicated in Figures 2(a) and 2(b), significant levels of ROS were formed in high glucose-treated ARPE-19 cells, compared with low-glucose culture conditions, for 48 hours. The quantitative data is shown in Figure 2(c), and it could be observed that ROS increased by approximately 40% in high glucose-stimulated cells vs. low-glucose conditions.

To investigate whether inflammasome was activated in ARPE-19 cells by high-glucose culture conditions, the mRNA levels of inflammasome marker genes, such as NLRP3, ASC, and caspase-1, were determined. As shown in Figures 3(a)–3(c), NLRP3 mRNA levels increased by over 85%, ASC mRNA levels increased by over 65%, and caspase-1 mRNA levels increased by over 60% in 48 hours under high-glucose culture conditions, when compared with the low-glucose culture medium.

3.3. N-Acetylcysteine Attenuates High Glucose-Induced Inflammatory Response in RPE Cells. To verify the role of ROS in stimulating proinflammatory cytokine production and inflammasome activation, ARPE-19 cells were treated with or without 2.5 mM of N-acetylcysteine (NAC), which is an antioxidant, combined with high- or low-glucose culture medium for 48 hours. As shown in Figures 4(a) and 4(b), 2.5 mM of NAC coculture could significantly attenuate the mRNA expression level of high glucose-induced proinflammatory cytokines IL-18 and IL-1β, particularly IL-1β, which was almost entirely abolished by NAC. At the protein level (Figures 4(c) and 4(d)), NAC could significantly block IL-18 and IL-1β production (P < 0.01). Similarly, 2.5 mM of NAC could also significantly ameliorate high glucose-induced inflammasome activation. As presented in Figures 4(e)–4(g), NAC could almost abolish the high glucose-induced mRNA expression of both NLRP3 and ASC and significantly attenuate caspase-1 mRNA expression.

3.4. The Knockdown of NLRP3 Expression Attenuates High Glucose-Induced RPE Cell Inflammatory Response in ARPE-19 Cells. Next, the association between inflammasome activation and proinflammatory cytokine expression and production was further tested. To do this, the siRNA knockdown approach was used to reduce NLRP3 expression, and its effects on IL-18 and IL-1β gene expression and protein
formation were tested. As shown in Figure 5(a), the specific siRNA that targeted NLRP3 significantly silenced NLRP3 expression, as determined by mRNA levels, but not by scrabbling siRNA. As shown in Figures 5(b) and 5(c), as expected, both ASC and caspase-1 mRNA expression levels were significantly reduced in high glucose-induced ARPE-19 cells after silencing NLRP3 gene expression. In addition, following siRNA-based NLRP3 gene silencing, it was observed that the

| Table 1: Primers used for the PCR gene. |
|----------------------------------------|
| **Forward, 5′-3′**                      | **Reverse, 5′-3′**                      |
| β-Actin                                | CGTTGAACCTGCGGTGGGTAG                   |
| NLRP3                                  | GCTTCAGTCCCACACACAG                     |
| ASC                                    | AGGCTGGTGTGAAACTGAAG                    |
| Caspase-1                               | TGCACAGACATACATACAGTTTC                 |
| IL-18                                   | ATAGAGGCCGATTTCTTTG                    |
| IL-1β                                   | TGTTGGTGAGAGATTCTGT                     |

Figure 1: The high-glucose condition decreased cell viability and induced proinflammatory cytokine IL-18 and IL-1β production in ARPE-19 cells. ARPE-19 cells were treated with high or low glucose for 48 hours. (a) Cell viability determined by the CCK-8 assay in the low-glucose group, high-glucose group, high-glucose+H2S group, and high-glucose+NAC group. (b) Protein levels of IL-18 detected by ELISA. (c) Protein levels of IL-1β detected by ELISA. (d) The mRNA expression levels of IL-18 detected by RT-PCR. (e) IL-1β mRNA levels measured by RT-PCR. Data were presented as the mean ± standard error (SE) of three independent experiments. *P < 0.05 and **P < 0.01, compared between the low-glucose and high-glucose groups (low glucose: 5.5 mM, high glucose: 25 mM).
mRNA expression of both IL-18 (Figure 5(d)) and IL-1β (Figure 5(e)) and the protein production of IL-18 (Figure 5(f)) and IL-1β (Figure 5(g)) were significantly reduced in high glucose-treated conditions, compared with scrabble siRNA-treated cells.

3.5. H₂S Decreases High Glucose-Induced ROS Production, NLRP3 Inflammasome Activation, and Inflammatory Cytokine Production in ARPE-19 Cells.

Finally, the effect of H₂S on high glucose-induced ROS production was investigated. Intrudingly, 200 μM of H₂S could completely abolish high glucose-induced ROS production, as determined by DCF fluorescence (Figures 6(a)–6(c)). Furthermore, 200 μM of H₂S could also completely abolish high glucose-induced NLRP3, ASC, and caspase-1 mRNA expression levels (Figures 6(d)–6(f)). Furthermore, H₂S also led to the significant attenuation of proinflammatory cytokine mRNA expression and protein secretion (Figures 7(a)–7(d)).

4. Discussion

The present study is aimed at understanding the responses and mechanisms of human RPE cells, that is, the ARPE-19 cell line, under high-glucose conditions with or without extracellular H₂S treatment. It was found that (1) high glucose could trigger a significant cell apoptosis and inflammatory response, as evidenced by the increase in ROS formation and NLRP3 inflammasome activation, (2) extracellular H₂S addition could attenuate high glucose-induced cell apoptosis and inflammatory response, and (3) the protection provided by H₂S was through blocking ROS formation and NLRP3 inflammasome activation.
Hyperglycemia has been implicated as an important contributing factor in DR progression through damaging the retinal microvasculature, resulting in retinal structure and function disorder [29]. In the present study, according to cell viability, inflammatory cytokines, intracellular ROS formation, and NLRP3 activation analysis, it was found that high glucose could significantly increase inflammation and apoptosis in ARPE-19 cells. A study conducted by Shen and Rong [29] indicated that mitochondrial ROS production was solely upstream responsible for high glucose-induced cell injury. Moreover, ROS has been considered to play a pivotal role in the activation of NLRP3 in the inflammasome, such as those reported in a rat model and HRMECs [14]. In 2002, the group of Prof. Tschopp described a multiprotein complex able to oligomerize and activate inflammatory caspases leading to the processing of IL-1β and IL-18. This complex was named NLR PYD-containing protein 3 (NLRP3) inflammasome [30]. In an excellent agreement, the present data revealed that hyperglycemia could induce the activation of the NLRP3 inflammasome and ultimately lead to the increased expression of IL-1β and IL-18. In addition, the silencing of NLRP3 gene expression through the siRNA approach could significantly ameliorate the high glucose-induced activation of IL-1β and IL-18. IL-1β is an inducible cytokine and is not generally expressed in healthy cells or tissue. Within the IL-1 cytokine family, IL-18 is most closely related to IL-1β and shares many common traits including cleavage by caspase-1 to a biologically active mature protein of ∼17 kDa that is actively secreted from cells. They all belong to the IL-1 family, which have been demonstrated to have broad and similar proinflammatory activity [31]. Taken together, these findings strongly support the important role of the NLRP3 inflammasome in high glucose-induced inflammation and apoptosis in RPE cells, as well as in the entire visual system.

At the same time, oxidative stress plays a pivotal role in the pathogenesis of diabetes and its complications [32].

![Graph](image_url)
Figure 4: Continued.
ROS levels are markedly increased in various diabetic models and are parallel with enhanced cell injury. Devi et al. found that high glucose could induce more elevated oxidative stress and the apoptosis of retinal pericytes and that antioxidant NAC and azaserine could significantly attenuate high glucose-induced ROS formation and DNA damage [33]. Moreover, several studies have indicated that ROS could promote inflammation and cell apoptosis with the progression of DR [5, 34, 35]. Similarly, in the present study, it was also demonstrated that 25 mmol/L of NAC could almost abolish high glucose-stimulated NLRP3 inflammasome activation and downstream inflammatory factors. Collectively, these results suggest that ROS formation stimulated by high glucose could induce inflammation by activating the NLRP3 inflammasome in ARPE-19 cells.

Gasotransmitters are a group of gaseous molecules, with pleiotropic biological functions. These molecules include nitric oxide (NO), hydrogen sulfide (H₂S), and carbon monoxide (CO). Abnormal production and metabolism of these molecules have been observed in several pathological conditions [36]. Among them, H₂S has become recognized as an important signaling molecule throughout the body, contributing to many physiological and pathological processes [37], affecting the function and activity of intracellular and extracellular proteins through different metabolic pathways [38, 39]. The potential properties of H₂S in improving pathological processes have been originally tested using H₂S donors such as sodium hydrogen sulfide (NaHS), Na₂S, N-acetylcysteine, or Lawesson’s reagent. It has been shown that high concentrations of the H₂S donor NaHS promote the release of TNF-α and IL-1 from IFN-γ-stimulated U937 cells, in an NFκB-dependent manner. Furthermore, studies have suggested that it could cope with ischemic injury, elevated oxidative stress, cell apoptosis, and inflammation [40]. For instance, it has been found that H₂S plays an important role in the regulation of pancreatic beta cell function, insulin resistance, and diabetes complications [25]. Furthermore, it can lower oxidative stress and various markers of vascular inflammation in diabetes [41]. A recent study conducted by Zhou et al. demonstrated that H₂S could block diabetic nephropathy in a streptozotocin-induced diabetic rat experimental model via normalizing oxidative stress and inflammation, preventing mesangial cell proliferation, and inhibiting renin-angiotensin system activity [42]. In a model of streptozotocin-induced diabetes in rats, H₂S formation was significantly increased in homogenates of the pancreas and liver of diabetic animals, as compared to healthy animals, and insulin treatment of streptozotocin-challenged rats reversed the increase in H₂S-synthesizing activity [43].

RPE cells are part of the blood-retinal barrier and play a crucial role in the maintenance of the visual system. RPE cells can secrete PEDF, VEGF, and other inflammatory cytokines, which participate in numerous pathologic and physiologic processes [44]. Chronic hyperglycemia could trigger the damage of RPE cells, subsequently affect the blood-retinal barrier, and ultimately promote the pathogenic progression of DR [44]. Moreover, Parsanathan and Jain found that exogenous administration of NaHS, a H₂S donor, can significantly upregulate the genes involved in GSH biosynthesis under diabetic conditions [45]. Furthermore, Jain et al. also showed that H₂S upregulated the glutamate-cysteine ligase catalytic subunit (GCLC) and GSH in a monocyte cell exposed to high glucose levels [20]. Therefore, it could be speculated that H₂S
Figure 5: The knockdown of NLRP3 gene expression ameliorated high glucose-induced proinflammatory cytokine production and inflammasome activation. The gene expression levels of IL-18 and IL-1β and inflammasome markers NLRP3, ASC, and caspase 1 were measured in ARPE-19 cells with either NLRP3-siRNA or scrabbling siRNA transfection in low- and high-glucose culture for 48 hours. (a) NLRP3 mRNA; (b) ASC mRNA; (c) caspase-1 mRNA; (d) IL-18 mRNA; (e) IL-1β mRNA; (f) intracellular IL-18 protein levels; (g) intracellular IL-1β protein levels. Data were presented as the mean ± standard error (SE) of three independent experiments. *P < 0.05 and **P < 0.01, compared between the high-glucose and high-glucose+NLRP3 siRNA groups. There was no statistical significance between the glucose group and the NLRP3 siRNA group (low glucose: 5.5 mM, high glucose: 25 mM).
Figure 6: H$_2$S decreased high glucose-induced ROS production and inflammatory response in RPE cells. Intracellular ROS formation and inflammasome marker NLRP3, ASC, and caspase-1 expression were determined in ARPE-19 cells after 48 hours of culture under low and high glucose, with or without 200 μM of NaHS pretreatment. (a) The ROS production indicated by FACs in high-glucose culture; (b) the ROS production indicated by FACs in high-glucose+NaHS culture; (c) a bar graph of average results from the three individual experiments; (d) NLRP3 mRNA; (e) ASC mRNA; (f) caspase-1 mRNA. Data were presented as the mean ± standard error (SE) of three independent experiments. *$P < 0.05$ and **$P < 0.01$, compared between the high-glucose and high-glucose+H$_2$S groups (low glucose: 5.5 mM, high glucose: 25 mM).
plays a protective role in the retina and DR by inhibiting ROS formation and inflammatory response at least partially through RPE cells, as reflected by a significantly increased level of GSH and reduced levels of proinflammatory cytokines IL-1β and IL-18, the decreased expression of NLRP3 inflammasome, and DCF fluorescence, which is a ROS marker. In addition, a significant reduction in IL-1β and IL-18 could be achieved after blocking either the ROS or the NLRP3 inflammasome complex, suggesting that the mechanism of H₂S in attenuating high glucose-induced inflammation is mediated through the amelioration of the ROS-NLRP3 inflammasome pathway.
5. Conclusions

In conclusion, the present study revealed that high glucose-stimulated RPE cell IL-18 and IL-1β secretion was induced through the activation of NLRP3 and ROS production. H2S can reduce the expression of IL-18 and IL-1β in RPE cells likely via the inhibition of the ROS-inflammasome pathway. These findings may support the potential therapeutic role of H2S in DR.

Abbreviations

DR: Diabetic retinopathy  
RPE: Retinal pigment epithelium  
H2S: Hydrogen sulfide  
ROS: Reactive oxygen species  
NAC: Antioxidant N-acetylcysteine  
PKC: Polyl pathway and protein kinase C  
VEGF: Vascular endothelial growth factor  
IGF-1: Insulin-like growth factor-1  
AGEs: Advanced glycation end products  
RAAS: Renin-angiotensin-aldosterone system  
HRMECs: Human retinal microvascular endothelial cells  
CSE: Cysteine by cystathionine γ-lyase  
GSH: Glutathione  
H2O2: Hydrogen peroxide  
NaHS: Sodium hydrosulfide  
FBS: Fetal bovine serum  
ATCC: American Type Culture Collection  
DCF: Dichlorofluorescein  
FACS: Fluorescence-activated cell sorting  
PCR: Polymerase chain reaction  
SE: Standard error  
ANOVA: Analysis of variance  
CSTC: Chongqing Key Laboratory of Ophthalmology.

Data Availability

All data generated or analyzed during the present study are included in this published article. More details are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

This work was supported by the fund projects of the National Natural Science Foundation of China (81870673), the Key Research Project of the Health Bureau of Chongqing (2011-1-029), and the special fund of the Chongqing Key Laboratory of Ophthalmology (CSTC).

References

[1] J. Cai and M. Boulton, “The pathogenesis of diabetic retinopathy: old concepts and new questions,” Eye, vol. 16, no. 3, pp. 242–260, 2002.
[2] A. M. Abu El-Asrar, E. Midena, M. Al-Shabrawey, and G. Mohammad, “New developments in the pathophysiology and management of diabetic retinopathy,” Journal Diabetes Research, vol. 2013, article 424258, 2 pages, 2013.
[3] J. Cunha-Vaz, L. Ribeiro, and C. Lobo, “Phenotypes and biomarkers of diabetic retinopathy,” Progress Retinal and Eye Research, vol. 41, pp. 90–111, 2014.
[4] J. M. Tarr, K. Kaul, M. Chopra, E. M. Kohner, and R. Chibber, “Pathophysiology of diabetic retinopathy,” ISRN Ophthalmology, vol. 2013, Article ID 343560, 13 pages, 2013.
[5] Y. Wu, L. Tang, and B. Chen, “Oxidative stress: implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives,” Oxidative Medicine and Cellular Longevity, vol. 2014, Article ID 752387, 12 pages, 2014.
[6] M. Williams, R. E. Hogg, and U. Chakravarthy, “Antioxidants and diabetic retinopathy,” Current Diabetes Reports, vol. 13, no. 4, pp. 481–487, 2013.
[7] L. Rochette, M. Zeller, Y. Cottin, and C. Vergely, “Diabetes, oxidative stress and therapeutic strategies,” Biochimica et Biophysica Acta (BBA) - General Subjects, vol. 1840, no. 9, pp. 2709–2729, 2014.
[8] E. Moran, L. Ding, Z. Wang et al., “Protective and antioxidant effects of PPARα in the ischemic retina,” Investigative Ophthalmology & Visual Science, vol. 55, no. 7, p. 4568, 2014.
[9] S. S. Cao and R. J. Kaufman, “Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease,” Antioxidants & Redox Signaling, vol. 21, no. 3, pp. 396–413, 2014.
[10] M. T. Lin and M. F. Beal, “Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases,” Nature, vol. 443, no. 7113, pp. 787–795, 2006.
[11] K. Schroder, R. Zhou, and J. Tschopp, “The NLRP3 inflammasome: a sensor for metabolic danger?,” Science, vol. 327, no. 5963, pp. 296–300, 2010.
[12] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, “A role for mitochrondia in NLRP3 inflammasome activation,” Nature, vol. 469, no. 7329, pp. 221–225, 2010.
[13] Y. H. Youm, A. Adjijang, B. Vandanmagsar, D. Burk, A. Ravussin, and V. D. Dixit, “Elimination of the NLRP3/ASC inflammasome protects against chronic obesity-induced pancreatic damage,” Endocrinology, vol. 152, no. 11, pp. 4039–4045, 2011.
[14] W. Chen, M. Zhao, S. Zhao et al., “Activation of the TXNIP/NLRP3 inflammasome pathway contributes to inflammation in diabetic retinopathy: a novel inhibitory effect of minocycline,” Inflammation Research, vol. 66, no. 2, pp. 157–166, 2017.
[15] N. Shibuya, S. Koike, M. Tanaka et al., “A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells,” Nature Communications, vol. 4, no. 1, p. 1366, 2013.
[16] F. N. Salloum, “Hydrogen sulfide and cardioprotection—mechanistic insights and clinical translatability,” Pharmacol & Therapeutics, vol. 152, pp. 11–17, 2015.
[17] S. Shimada, M. Fukai, K. Wakayama et al., “Hydrogen sulfide augments survival signals in warm ischemia and reperfusion...
of the mouse liver,” Surgery Today, vol. 45, no. 7, pp. 892–903, 2015.

[18] B. S. Kasinath, “Hydrogen sulfide to the rescue in obstructive kidney injury,” Kidney International, vol. 85, no. 6, pp. 1255–1258, 2014.

[19] Y. Kimura and H. Kimura, “Hydrogen sulfide protects neurons from oxidative stress,” The FASEB Journal, vol. 18, no. 10, pp. 1165–1167, 2004.

[20] S. K. Jain, L. Huning, and D. Micinski, “Hydrogen sulfide upregulates glutamate-cysteine ligase catalytic subunit, glutamate-cysteine ligase modifier subunit, and glutathione and inhibits interleukin-1β secretion in monocytes exposed to high glucose levels,” Metabolic Syndrome and Related Disorders, vol. 12, no. 5, pp. 299–302, 2014.

[21] B. Olas, “Chapter six - hydrogen sulfide as a “double-faced” compound: one with pro- and antioxidant effect,” Advances in Clinical Chemistry, vol. 78, pp. 187–196, 2017.

[22] S. K. Jain, R. Bull, J. L. Rains et al., “Low levels of hydrogen sulfide in the blood of diabetes patients and streptozotocin-treated rats causes vascular inflammation,” Antioxidants & Redox Signaling, vol. 12, no. 11, pp. 1333–1337, 2010.

[23] K. Shahzad, F. Bock, W. Dong et al., “Nirp3-inflammasome activation in non-myeloid-derived cells aggravates diabetic nephropathy,” Kidney International, vol. 87, no. 1, pp. 74–84, 2015.

[24] G. G. Wang, Q. Y. Chen, W. Li, X. H. Lu, and X. Zhao, “Ginkgolide B increases hydrogen sulfide and protects against endothelial dysfunction in diabetic rats,” Croatian Medical Journal, vol. 56, no. 1, pp. 4–13, 2015.

[25] C. Szabo, “Roles of hydrogen sulfide in the pathogenesis of diabetes mellitus and its complications,” Antioxidants & Redox Signaling, vol. 17, no. 1, pp. 68–80, 2012.

[26] K. Sakamoto, Y. Suzuki, Y. Kurauchi, A. Mori, T. Nakahara, and K. Ishii, “Hydrogen sulfide attenuates NMDA-induced neuronal injury via its anti-oxidative activity in the rat retina,” Experimental Eye Research, vol. 120, pp. 90–96, 2014.

[27] K. J. Livak and T. D. Schmittgen, “Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method,” Methods, vol. 25, no. 4, pp. 402–408, 2001.

[28] X. L. Chen, X. D. Zhang, Y. Y. Li, X. M. Chen, D. R. Tang, and R. J. Ran, “Involvement of HMGB1 mediated signalling pathway in diabetic retinopathy: evidence from type 2 diabetic rats and ARPE-19 cells under diabetic condition,” The British Journal of Ophthalmology, vol. 97, no. 12, pp. 1598–1603, 2013.

[29] H. Shen and H. Rong, “Pterostilbene impact on retinal endothelial cells under high glucose environment,” International Journal of Clinical and Experimental Pathology, vol. 8, no. 10, pp. 12589–12594, 2015.

[30] F. Martino, K. Burns, and J. Tschopp, “The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proll-beta,” Molecular Cell, vol. 10, no. 2, pp. 417–426, 2002.

[31] L. A. Borthwick, “The IL-1 cytokine family and its role in inflammation and fibrosis in the lung,” Seminars in Immunopathology, vol. 38, no. 4, pp. 517–534, 2016.

[32] F. Giacco and M. Brownlee, “Oxidative stress and diabetic complications,” Circulation Research, vol. 107, no. 9, pp. 1058–1070, 2010.

[33] T. S. Devi, K.-I. Hosoya, T. Terasaki, and L. P. Singh, “Critical role of TXNIP in oxidative stress, DNA damage and retinal pericyte apoptosis under high glucose: implications for diabetic retinopathy,” Experimental Cell Research, vol. 319, no. 7, pp. 1001–1012, 2013.

[34] J. Kruk, K. Kubasik-Kladna, and H. Y. Aboul-Enein, “The role oxidative stress in the pathogenesis of eye diseases: current status and a dual role of physical activity,” Mini Reviews in Medicinal Chemistry, vol. 16, no. 3, pp. 241–257, 2015.

[35] R. A. Kowluru and P. S. Chan, “Oxidative stress and diabetic retinopathy,” Experimental Diabetes Research, vol. 2007, Article ID 43603, 12 pages, 2007.

[36] P. Fagone, E. Mazzon, P. Bramanti, K. Bendtzen, and F. Nicoletti, “Gasotransmitters and the immune system: mode of action and novel therapeutic targets,” European Journal of Pharmacology, vol. 834, pp. 92–102, 2018.

[37] J. L. Wallace and R. Wang, “Hydrogen sulfide-based therapeutics: exploiting a unique but ubiquitous gasotransmitter,” Nature Reviews. Drug Discovery, vol. 14, no. 5, pp. 329–345, 2015.

[38] H. Kimura, “The physiological role of hydrogen sulfide and beyond,” Nitric Oxide, vol. 41, pp. 4–10, 2014.

[39] B. Olas, “Hydrogen sulfide in signaling pathways,” Clinica Chimica Acta, vol. 439, pp. 212–218, 2015.

[40] O. Kabil, N. Moti, and R. Banerjee, “H2S and its role in redox signaling,” Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, vol. 1844, no. 8, pp. 1355–1366, 2014.

[41] P. Manna and S. K. Jain, “L-cysteine and hydrogen sulfide increase PI3P and AMPK/PAR2 expression and decrease ROS and vascular inflammation markers in high glucose treated human U937 monocytes,” Journal of Cellular Biochemistry, vol. 114, no. 10, pp. 2334–2345, 2013.

[42] X. Zhou, Y. Feng, Z. Zhan, and J. Chen, “Hydrogen sulfide alleviates diabetic nephropathy in a streptozotocin-induced diabetic rat model,” The Journal of Biological Chemistry, vol. 289, no. 42, pp. 28827–28834, 2014.

[43] M. Yusuf, B. T. Kwong Huat, A. Hsu, M. Whitman, M. Bhatia, and P. K. Moore, “Streptozotocin-induced diabetes in the rat is associated with enhanced tissue hydrogen sulfide biosynthesis,” Biochemical and Biophysical Research Communications, vol. 333, no. 4, pp. 1146–1152, 2005.

[44] R. Simo, M. Villarroel, L. Corraliza, C. Hernandez, and M. Garcia-Ramirez, “The retinal pigment epithelium: something more than a constituent of the blood-retinal barrier – implications for the pathogenesis of diabetic retinopathy,” Journal of Biomedicine & Biotechnology, vol. 2010, Article ID 190724, 15 pages, 2010.

[45] R. Parsanathan and S. K. Jain, “Hydrogen sulfide increases glutathione biosynthesis, and glucose uptake and utilisation in C2C12 mouse myotubes,” Free Radical Research, vol. 52, no. 2, pp. 288–303, 2018.