Hydrogen Sulfide Ameliorates High Glucose-induced Inflammation Through SIRT1-mTOR/NF-κB Signaling Pathway in HT-22 cells

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Research

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Abstract

Background: Hyperglycemia-induced neuroinflammation promotes the progression of diabetic encephalopathy (DE). Hydrogen sulfide (H_2S) exerts anti-inflammatory and neuroprotective activities against neurodegenerative diseases. However, its role in hyperglycemia-induced neuronal inflammation has not been investigated. Herein, we examined the effects and its related signaling pathway of H_2S on inflammatory response in high glucose-treated HT-22 cells.

Methods: A hippocampal neuronal cell line, HT-22, was used as an *in vitro* model to explore the function of H_2S on inflammatory response triggered by high glucose. A dicyanoisophorone-based near-infrared fluorescent probe (NIR-NP) was synthesized to detect H_2S levels in HT-22 cells. Western blotting, immunofluorescence and real time-qPCR were carried out to study the mechanism of action for H_2S.

Results: We found that high glucose (85 mM) decreased the level of endogenous H_2S and the expression of cystathionine-β-synthase (CBS) which is the main enzyme for H_2S production in the brain. Sodium hydrosulfide (NaHS, a H_2S donor) or S-adenosylmethionine (SAMe, an allosteric activator of CBS) administration restored high glucose-induced downregulation of CBS and H_2S levels. Importantly, high glucose upregulated the level of pro-inflammatory factors (IL-1β, IL-6, TNF-α) in HT-22 cells. Treatment with NaHS or SAMe alleviated this enhanced transcription of these pro-inflammatory factors, suggesting that H_2S might ameliorate high glucose-induced inflammation in HT-22 cells. We also found that high glucose reduced SIRT1 protein levels. SIRT1 reduction elevated the level of p-mTOR, p-NF-κB and pro-inflammatory factors, which were restored by resveratrol (a SIRT1 agonist). These results suggested that SIRT1 might be an upstream mediator of mTOR/NF-κB signaling pathway. Furthermore, NaHS or SAMe treatment reversed the expression of SIRT1, mTOR and NF-κB under high glucose conditions.

Conclusions: Our study revealed that high glucose decreased CBS to reduce the production of H_2S, which in turn decreased the expression of SIRT1. The reduction of SIRT1 activated mTOR/NF-κB signaling to promote inflammation. Given that promoting H_2S production using NaHS or SAMe can reverse high glucose-induced inflammatory response, our study might shed light on the prophylactic treatment of DE.

Background

Diabetic encephalopathy (DE) is recognized as a complex and elusive complication of diabetes nowadays [1]. Patients with DE exhibit progressive alterations in brain structures and cognitive decline. These clinical manifestations are primarily caused by hyperglycemia-induced neurotoxicity [2]. Although multiple pathophysiological processes are involved in the progression of DE, accumulating evidence suggests that neuroinflammation plays a critical role in neurodegeneration induced by hyperglycemia [3, 4]. In diabetic animals, the expression of neuroinflammatory cytokines is significantly increased in the hippocampus, an important brain region for cognition [3]. High glucose increases pro-inflammatory response in the immune cells such as microglia and astrocyte in the central nervous system [5, 6].

However, in neurons, changes triggered by high glucose in the expression of inflammatory cytokines and associated signaling molecules are less reported. The underlying molecular mechanisms about how hyperglycemia activates inflammation in the brain remain further elucidate.

Hydrogen sulfide (H_2S) is a noteworthy endogenous gasotransmitter which participates in regulating various pathophysiological processes [7]. During the progression of diabetes, the H_2S levels declined gradually in the patients' plasma [8, 9]. In line with these clinical findings, preclinical research revealed that hyperglycemia reduced H_2S biosynthesis and increased its degradation in rodent models of diabetes [10, 11]. While, supplementation of H_2S donor sodium hydrosulfide (NaHS) protected hyperglycemia-induced myocardial tissue damage *in vivo* and *in vitro* [12]. In the central nervous system, the production of endogenous H_2S is mainly catalyzed by cystathionine-β-synthase (CBS) [13]. Exogenous H_2S inhibited the expression of pro-inflammatory factors and decreased the generation of oxidative indicator reactive oxygen species in the mouse model of Alzheimer's disease [14, 15]. However, it has not been fully investigated the effects and mechanisms of H_2S on the inflammation in neurons under hyperglycemia.

Recent research has shown that H_2S regulated inflammation-associated signaling molecules, such as silent information regulator-1 (SIRT1), mammalian target of rapamycin (mTOR) and nuclear factor-κB (NF-κB). H_2S upregulated the expression of SIRT1 to prevent homocysteine-induced neurotoxicity in PC12 cells [16]. H_2S increased SIRT1 levels in the rat hippocampus, which reduced chronic mild stress-induced depressive-like behavior [17]. However, it is unknown if SIRT1 and its downstream signaling in the neuron are interrupted by high glucose. mTOR is a crucial protein kinase involved in regulating multiple diseases including cancer, metabolic and neurological diseases. mTOR signaling was dysregulated in type 2 diabetes as well as inflammation [18]. Upregulation of SIRT1 inhibited the expression of mTOR and subsequently attenuated inflammation in bleomycin-induced scleroderma mice and high-fat-diet mice [19, 20], suggesting SIRT1 might be an upstream signaling molecule of mTOR. However, SIRT1-mTOR singling pathway has not been investigated in neurons with high glucose. Activation of mTOR modulated its downstream ubiquitous transcriptional factor NF-κB to facilitate the production of pro-inflammatory cytokines [21, 22]. H_2S plays an inhibitory role in lipopolysaccharide (LPS)-triggered inflammatory response via blocking the transactivation of NF-κB in endothelial cells[23]. Collectively, we predicted that H_2S might modulate inflammation in neuronal cells by inhibiting the activated SIRT1/mTOR/NF-κB signaling pathway.

In the current study, we applied a neuronal cell line HT-22, which was widely used as an *in vitro* model of neurodegenerative disease [24, 25], to explore the function of high glucose on H_2S and its endogenous synthase CBS, as well as pro-inflammatory cytokines expression and SIRT1/mTOR/NF-κB signaling pathway. Furthermore, we examined whether NaHS or S-adenosylmethionine (SAMe, an allosteric activator of CBS) could attenuate high glucose-induced inflammatory effect.

Methods

Cell line and reagents

The mouse hippocampal neuronal cell line HT-22 cells were kindly gifted from the Research Center for Neurobiology of Xuzhou Medical University (Xuzhou, China). NaHS and SAMe (97% purity) was purchased from Aladdin Reagent Co., Ltd (Shanghai, China) and MedChemExpress (Monmouth Junction, NJ, USA), respectively. Resveratrol and glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against CBS and β-actin were obtained from Santa Cruz *Biotechnology* Inc. (Santa Cruz, CA, USA) and ZSGB-BIO (Beijing, China), respectively. Antibodies against SIRT1, p-mTOR, mTOR, p-NF-κB p65 and NF-κB p65 were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). Unless specifically indicated, all other reagents were purchased commercially.

Synthesis of H_2S probe NIR-NP

A dicyanoisophorone-based near-infrared fluorescent probe (NIR-NP) for detection of H_2S was synthesized as described in the previous study [26] and its structure was verified by ^1H NMR and HRMS. The spectroscopic properties of NIR-NP probe such as selectivity, sensitivity and stability were validated using a Hitachi F-4600 fluorescence spectrophotometer.

Cell culture and treatments

The culture of HT-22 cells was conducted in DMEM medium containing 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin in an incubator (37 ℃, 5% CO_2). HT-22 cells were grown in 96-well plate (5×10^3 cells/well) for 24 h and incubated with glucose, SAMe, NaHS, resveratrol and NIR-NP at a range of concentrations for another 24 h, respectively. Then the cells were incubated with MTT (0.1 mg/well) at 37 ℃ for 4 h. The Microplate reader (ELX808IU, Bio-tek Instruments Inc., USA) was used to detect the optical density at 550 nm.

According to previous reports [27-30] and the results of MTT assay (Supplementary Figure S1), we selected glucose, SAMe, NaHS and resveratrol at a concentration of 85 mM, 0.2 mM, 250 μM and 10 μM to treat HT-22 cells, respectively, and divided them into the following five groups: control group (25 mM glucose), high glucose (HG) group (85 mM glucose), HG + NaHS group (85 mM glucose + 250 μM NaHS), HG + SAMe group (85 mM glucose + 0.2 mM SAMe) and HG + resvertatrol (RES) group (85 mM glucose + 10 μM resveratrol).

Cell imaging

HT-22 cells were cultured on coverglass (Lab-TekH II Chambered Coverglass, Nale Nunc, Naperville, USA). After being treated with high glucose, SAMe, NaHS or resveratrol as above mentioned, HT-22 cells were incubated with 10 μM NIR-NP for 30 min and Na_2S (50 μM and 100 μM) for following 20 min. The

Olympus FV1000 confocal laser scanning microscope was used to obtain confocal fluorescence images. Images and data were analyzed using Olympus software (FV10-ASW).

The detection of H_2S levels in HT-22 cells using NIR-NP

The levels of H_2S in HT-22 cells were examined using NIR-NP according to the reported method [31]. Cells were treated with high glucose and/or other agents and then collected in PBS buffer. Cells were homogenized and centrifuged at 12,000 *g* at 4 ℃ for 15 min. The Pierce BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China) was used to determine total protein concentrations of the collected supernatant. The H_2S content in the treated cells was determined using supernatant spiked with Na_2S solution at a series of concentrations as the internal criterion. To obtain zero point, $ZnCl_2$ was added to scavenge the endogenous H_2S within the sample. In Eppendorf tubes, 20 μL homogenates supernatant and 1 μL 1.0 mM NIR-NP probe were mixed with 69 μL PBS, which was spiked with 0, 0.4, 0.8,1.2, 1.6 μL of 100 μM Na_2S solution and double distilled water (10, 9.6, 9.2, 8.8, 8.4 μL) correspondingly. After incubation at 37 ℃ for 20 min, the fluorescence intensity was detected using the Hitachi F4600 Fluorescence Spectrophotometer. Each sample was measured at least three times in parallel. According to the calibration curve of Na_2S, the concentration of each sample was calculated and expressed as μmol/g protein.

Real-time quantitative polymerase chain reaction (RT-qPCR)

The TRIzol reagent (Invitrogen Co., Carlsbad, CA, USA) was used for the extraction of total RNA. The determination of total RNA concentration was performed on NanoDrop 1000 spectrophotometer (Thermo Scientific). ReverTra Ace qPCR RT kit (TOYOBO CO., LTD.) was used to synthesize cDNA. The forward and reverse primers sequences of IL-1β, TNF-α, IL-6 and β-actin (Sangon Biotech Co. Ltd., Shanghai, China) were presented in Table 1. 10 μL reaction solution including SYBR Green MIX, cDNA, forward primer, reverse primer and RNase-free water was centrifuged and subject to LightCycle 480 (Roche Applied Science). LightCycler 480 software was used to determine the relative mRNA levels.

Western BlottingA Pierce BCA Protein Assay Kit was used to determine the supernatant protein concentrations of HT-22 cell lysis buffer. After being electrophoresed in SDS-PAGE gels, the separated proteins were transferred to nitrocellulose membranes, which were blocked at 37 ℃ for 2 h in Tris-buffered saline with 5% BSA and 0.05% Tween 20 and incubated with the primary antibodies against CBS, SIRT1, mTOR, p-mTOR, NF-κB p65, p-NF-κB p65 and β-actin (all antibodies were diluted at 1:1000) at 4 ℃ overnight, respectively. Subsequently, the membranes were incubated with the secondary horseradish peroxidase-linked anti-rabbit (1:10000) or anti-mouse (1:10000) antibodies (ZSGB-BIO, Beijing, China) at 37 ℃ for 1 h. After the membranes were washed with TBST, chemiluminescent reagent was applied and the images were captured using Odyssey infrared fluorescence imaging system.ImmunofluorescenceHT-22 cells were cultured on glass coverslips in a 24-well plate (1.5×10^4

cells/well). After being rinsed with PBS, HT-22 cells were fixed in 4% paraformaldehyde for 15 min, and then penetrated with 0.5% Triton X-100 (PBS) for 5 min and blocked at room temperature in 1 mL of PBS solution containing 5% FBS for 1 h. After blocking, HT-22 cells were incubated at 4 ℃ with the primary antibodies against CBS, SIRT1, p-mTOR and p-NF-κB p65 (All antibodies were diluted at 1:200) overnight and then incubated at room temperature in the dark with 300 μL Alexa Fluor-labeled fluorescent anti-rabbit (1:200) or anti-mouse (1:200) antibodies for 1 h. 300 μL DAPI solution (1 mg/mL) were then applied for the following 5 min. The coverslips were placed on a slide with antifade mounting medium. Images were acquired using the OLYMPUS upright fluorescence microscope and analyzed with Image J.Statistical AnalysisAll results are analyzed by SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). The intergroup differences were assessed by one-way ANOVA accompanied by LSD test or Dunnett's T3 post hoc analysis. Data from all experiments were presented as mean ± SD (n = 3). $P < 0.05$ represented a statistical significance.

Table 1
Primer sequences for the RT-qPCR analysis.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
IL-1β	GCAGAGCACAAGCCTGTCTTCC	ACCTGTCTTGGCCGAGGACTAAG
TNF-α	GCGACGTGGAACTGGCAGAAG	GCCACAAGCAGGAATGAGAAGAGG
IL-6	AGGAGTGGCTAAGG ACCAAGACC	CTGACCACAGTGAGGAATGTCCAC
β-actin	CCCATCTATGAGGGTTACGC	TTTAATGTCACGCACGATTTC

Results

The efficacy of NIR-NP to detect endogenous H_2S in HT-22 Cells

The compound NIR-NP was successfully synthesized and confirmed by ^1H NMR (Figure S2) and HRMS (Figure S3). The probe exhibited excellent properties including an off-on response to H_2S (Figure S4), linearity (Figure S5), fast response time (Figure S6), high selectivity for H_2S over other analytes (Figure S7) and pH stability (Figure S8). We then examined the effect of NIP-NP on the cell viability using MTT assay. Different concentrations of NIP-NP (5, 10, 20, 40, and 80 μM) exhibited no significant cytotoxicity (Figure 1A). These results suggest that the NIP-NP probe is less toxic and biocompatible for intracellular bioimaging. As shown in Figure 1B, when HT-22 cells were incubated with NIR-NP and Na_2S (H_2S donor), obvious red fluorescence in the cytoplasm was observed. When Na_2S concentration was increased, the fluorescent intensity was enhanced, suggesting that NIR-NP was able to detect the H_2S levels in HT-22 cells.

NaHS and SAMe prevented high glucose-induced reduction of H_2S and CBS levels in HT-22 cells

The probe NIR-NP was used to detect H_2S contents in HT-22 cells. The H_2S level in the HG group was evidently decreased than that of the control group ($P < 0.001$, Figure 2A). SAMe or NaHS application at 30 min prior to high glucose treatment significantly increased H_2S levels compared with the HG group (both $P < 0.001$). The results indicated that the SAMe and NaHS improved the H_2S level, which was reduced by the high glucose.

CBS is an important enzyme to catalyze H_2S [13]. Next, we found that high glucose reduced CBS protein levels in HT-22 cells (Figure 2B, $P < 0.001$).The CBS level was obviously elevated in HG + SAMe and HG + NaHS groups compared with the HG group (Figure 2B, both $P < 0.001$). The results of the quantification of immunofluorescent intensity were consistent with this finding and showed the alteration of CBS in the HT-22 cells (Figure 2C, $P < 0.01$, $P < 0.001$).

High glucose triggered inflammatory response via SIRT1/mTOR/NF-κB p65 pathway

Studies have shown that SIRT1 negatively modulates mTOR [19] and NF-κB [32]. Therefore, SIRT1 may serve as an important regulator of high glucose-mediated inflammatory signaling and pro-inflammatory cytokines. RT-qPCR results showed that high glucose upregulated the mRNA expression of IL-6, IL-1β and TNF-α in HT-22 cells (Figure 3, all $P < 0.001$), while resveratrol (an agonist of SIRT1) inhibited the increase of these pro-inflammatory cytokines (Figure 3, all $P < 0.001$).

Furthermore, both western blot and immunofluorescent results showed that resveratrol rescued the downregulation of SIRT1 in high glucose-treated HT-22 cells (Figure 4A, $P < 0.01$, $P < 0.001$). Resveratrol also alleviated high glucose-induced phosphorylation of mTOR (Figure 4B, both $P < 0.01$) and NF-κB p65 (Figure 4C, $P < 0.001$, $P < 0.01$), suggesting that SIRT1 might be an important mediator of high glucose-induced pro-inflammatory factors production via activating the mTOR/NF-κB p65 signaling pathway.

H_2S prevented high glucose-induced increase of pro-inflammatory cytokines

It is reported that the H_2S level is negatively associated with inflammation [33]. Following the finding that high glucose reduced H_2S level and increased pro-inflammatory response in neuronal HT-22 cells, we next examined if SAMe or NaHS can prevent these processes. RT-qPCR results showed that IL-6, IL-1β and TNF-α levels were significantly increased in high glucose-treated HT-22 cells (Figure 5, all $P < 0.001$). Importantly, the upregulation of pro-inflammatory cytokines was attenuated by either SAMe or NaHS

treatment (Figure 5, all $P < 0.001$), suggesting that increasing H_2S can prevent high glucose-induced neuroinflammation in HT-22 cells.

H_2S rescued high glucose-induced the alterations of SIRT1-mTOR/NF-κB signaling

It is reported that H_2S regulated SIRT1 expression in both *in vitro* and *in vivo* studies [16, 17]. Therefore SIRT1 and its associated mTOR/NF-κB signaling molecules were examined in response to SAMe or NaHS. SIRT1 level was significantly decreased in the HG group (Figure 6A, $P < 0.01$), while SAMe or NaHS restored the SIRT1 level in HT-22 cells (Figure 6A, both $P < 0.001$). Immunofluorescent analysis further confirmed this finding (Figure 6A, $P < 0.01$, $P < 0.05$). Furthermore, the phosphorylation of mTOR (Figure 6B, $P < 0.01$) and NF-κB p65 (Figure 6C, $P < 0.01$) were promoted by high glucose, which was repressed by the treatment of SAMe or NaHS (Figure 6B-C, mTOR, both $P < 0.001$; NF-κB p65, both $P < 0.01$). Total mTOR and NF-κB p65 proteins were not altered by either high glucose, SAMe or NaHS (Figure 6B-C, all $P > 0.05$). Immunofluorescent quantification analysis were consistent with these results (Figure 6B-C, mTOR, all $P < 0.001$; NF-κB p65, all $P < 0.01$).

Discussion

In this study, the NIR-NP probe was successfully synthesized and showed excellent selectivity and sensitivity for the detection of endogenous H_2S levels in HT-22 cells. Using this probe, we showed that high glucose reduced the H_2S levels in neurons. Furthermore, high glucose exposure decreased the level of CBS, SIRT1 and elevated the level of pro-inflammatory cytokines IL-6, IL-1β and TNF-α along with mTOR/NF-κB signaling activation. Importantly, NaHS or SAMe reversed the level of H_2S, CBS, pro-inflammatory cytokines and signaling molecules under high glucose conditions. As neuroinflammation occupies a critical position in the development of DE [34], our results might provide novel insight that H_2S decline and neuroinflammation in neurons are involved in the pathophysiology of DE.

DE is a complication that occurred in long-term diabetes [35]. The hyperglycemia-induced inflammation is an emerging factor that contributes to the progression of DE. In the brain, hyperglycemia-induced inflammatory response included microglia activation and increased pro-inflammatory cytokines production [36, 37]. In the present study, high glucose treatment is demonstrated to elevate the mRNA levels of pro-inflammatory cytokines in HT-22 cells directly. We further revealed that this increased inflammation was mediated through the downregulation of H_2S, as restoring H_2S levels using NaHS or SAMe attenuated the upregulation of pro-inflammatory cytokines induced by high glucose. These results are consistent with previous findings that H_2S attenuates LPS-induced inflammatory response in endothelial cells and microglia [23, 38]. Our previous preclinical research has also proved that H_2S attenuates hippocampal inflammation in the $AlCl_3$-induced mouse model of Alzheimer's disease [14].

Collectively, these findings suggest that the reduction of H_2S in neurons contributes to high-glucose induced neuroinflammation.

We also found that high glucose reduced H_2S synthesizing enzyme, CBS protein level in HT-22 neurons, while NaHS or SAMe restored CBS and endogenous H_2S levels. The endogenous H_2S is mainly regulated by three enzymes including CBS, CSE and 3-MST [39]. In the brain, CBS is the major enzyme to modulate the endogenous H_2S synthesis. NaHS treatment has been shown to restore H_2S production and CBS expression in a mouse model of intracerebral hemorrhage [40]. SAMe, a CBS activator, increased the H_2S synthesis in the central nervous system [28]. In the current study, our results showed that NaHS or SAMe upregulated CBS expression and restored high glucose-induced reduction of H_2S in neuronal cells. The reduced expression of CBS has been found in the brain of Alzheimer's disease patients [41]. Interestingly, impaired glycemia is associated with the progression from mild cognitive impairment to Alzheimer's disease [42]. Therefore, these findings suggest that enhancement of CBS is a potential target to increase H_2S to prevent hyperglycemia associated neurodegenerative disease.

Increasing evidence revealed that SIRT1, a NAD^+-dependent deacetylase, modulated inflammatory response. For example, inflammation stimulator, e.g. LPS, reduced the SIRT1 expression and activated pro-inflammatory cytokines levels in vascular endothelial cells [43]. The activation of SIRT1 facilitated the formation of facultative heterochromatin to silence pro-inflammatory genes, for instance, TNF-α and IL-1β in THP-1 promonocytes [44–46]. Here, we found that SIRT1 agonist, resveratrol prevented high glucose-induced pro-inflammatory cytokines expression in HT-22 neurons. Therefore, SIRT1 is an important mediator of glucose-induced neuroinflammation. Research has shown H_2S upregulated SIRT1 to protect neurotoxicity in PC12 cells induced by homocysteine [16]. SIRT1 mediated the roles of H_2S in attenuating cognitive impairment induced by chronic restraint stress in rats [47]. In the present work, we found that H_2S enhancer, SAMe and NaHS upregulated SIRT1 expression and downregulated pro-inflammatory cytokines expression in high glucose-exposed HT-22 cells. These results indicated that SIRT1 mediates the protection of H_2S against high glucose-triggered neuronal inflammation in HT-22 cells.

Previously, studies have shown that SIRT1 negatively regulated mTOR signaling in a variety of cells, such as MEFs cells, HeLa cells and cardiomyocytes [48, 49]. In the current work, we showed that SIRT1 mediated mTOR signaling in response to high glucose in neurons, evidenced by SIRT1 agonist resveratrol inhibited high glucose-induced phosphorylation of mTOR in HT-22 cells. High glucose-induced mTOR phosphorylation is decreased by the treatment of H_2S donor GYY4137 in cardiac H9c2 cells [50]. It is documented that inflammatory stress induced an mTOR activation in hepatoblastoma HepG2 cells [51]. Here, we have shown that both H_2S donor NaHS and the allosteric CBS activator SAMe treatment prevented mTOR activation induced by high glucose, suggesting that cellular H_2S supplementation may regulate SIRT1-mTOR to provide anti-neuroinflammation effects. NF-κB, a well-known nuclear transcription factor, regulates the mRNA levels of inflammation-related factors [52]. NF-κB is a downstream mediator of mTOR in immune cells [22]. Furthermore, enhancing SIRT1 expression using resveratrol suppressed NF-κB activation and inhibited the secretion of pro-inflammatory mediators in

macrophage RAW264.7 cells [32]. Therefore, NF-κB is a downstream molecule of SIRT1-mTOR for inflammatory response. Our results revealed that resveratrol or SAMe/NaHS restored high glucose-induced NF-κB activation in HT-22 cells. Overall, these findings suggest that the H_2S supplement might modulate SIRT1-mTOR-NF-κB signaling pathway and thus inhibit high glucose-induced pro-inflammatory cytokine expression.

Conclusions

In summary, our results have shown that high glucose activated inflammation through reducing H_2S and its synthesizing enzyme CBS in neurons. Conversely, restore H_2S levels inhibited inflammation activation. As inflammation is considered to promote neurodegenerative progression in DE, exogenous H_2S treatment or enhancing endogenous H_2S synthesis might prevent these inflammatory processes and delay neurodegeneration. Our findings laid the groundwork for a profound understanding of DE pathogenesis and provide new evidence for DE treatment.

Abbreviations

BSA: bovine serum albumin; CBS: cystathionine-β-synthase; CSE: cystathionine-γ-lyase; DE: diabetic encephalopathy; DMEM: dulbecco's modified eagle medium; FBS: fetal bovine serum; ^{1}H NMR: ^{1}H nuclear magnetic resonance; H_2S: hydrogen sulfide; HG: high glucose; HRMS: high resolution mass spectrometry; IL-1β: interleukin-1β; IL-6: interleukin-6; LPS: lipopolysaccharide; 3-MST: 3-mercaptopyruvate sulfurtransferase; mTOR: mammalian target of rapamycin; MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; Na_2S: sodium sulfide; NaHS: sodium hydrosulfide; NF-κB: nuclear factor-κB; PBS: phosphate-buffered saline; RES: resveratrol; RT-qPCR: real-time quantitative polymerase chain reaction; SAMe: S-adenosylmethionine; SIRT1: silent information regulator-1; TBST: tris-buffered saline Tween-20; TNF-α: tumor necrosis factor-α.

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this article and its supplementary information files.

Authors' contributions

YL conceived and designed the experiments; XL, PY, TX, JL, YC, XY and CY performed the experiments and analyzed the data; XL and PY wrote the manuscript; XC and YY contributed to editing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Figures

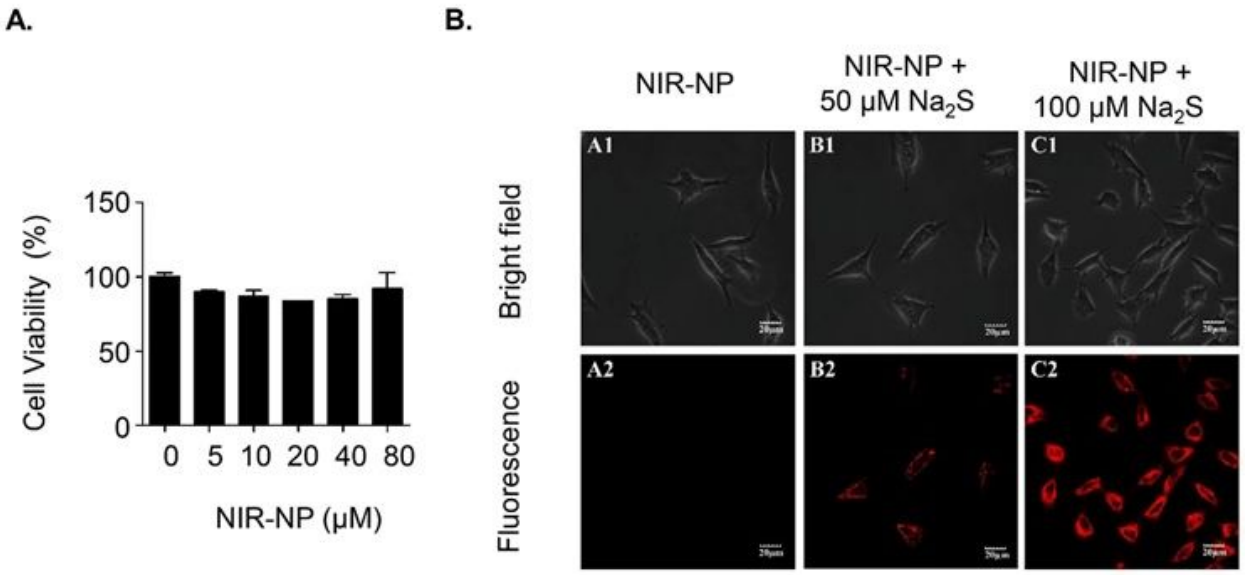

Figure 1

(A) Effects of NIR-NP on the cell viability of HT-22 cells. Cells were treated with a series of concentrations of NIR-NP (0, 5, 10, 20, 40, 80 μM) for 24 h and cell viability was estimated by MTT assay. Data are presented as mean ± SD. (n = 3). (B) Confocal fluorescence images in HT-22 cells. A1-A2: Cells were incubated with NIR-NP (10 μM) at 37 ℃ for 30 min. B1-B2: Cells were incubated with NIR-NP (10 μM) for 30 min and then with Na2S (50 μM) at 37 ℃ for 20 min. C1-C2: Cells were incubated with NIR-NP (10 μM) for 30 min and then with Na2S (100 μM) at 37 ℃ for 20 min. A1, B1, and C1 are bright-field images; A2, B2, and C2 are the corresponding fluorescence images. All scale bars were 20 μm.

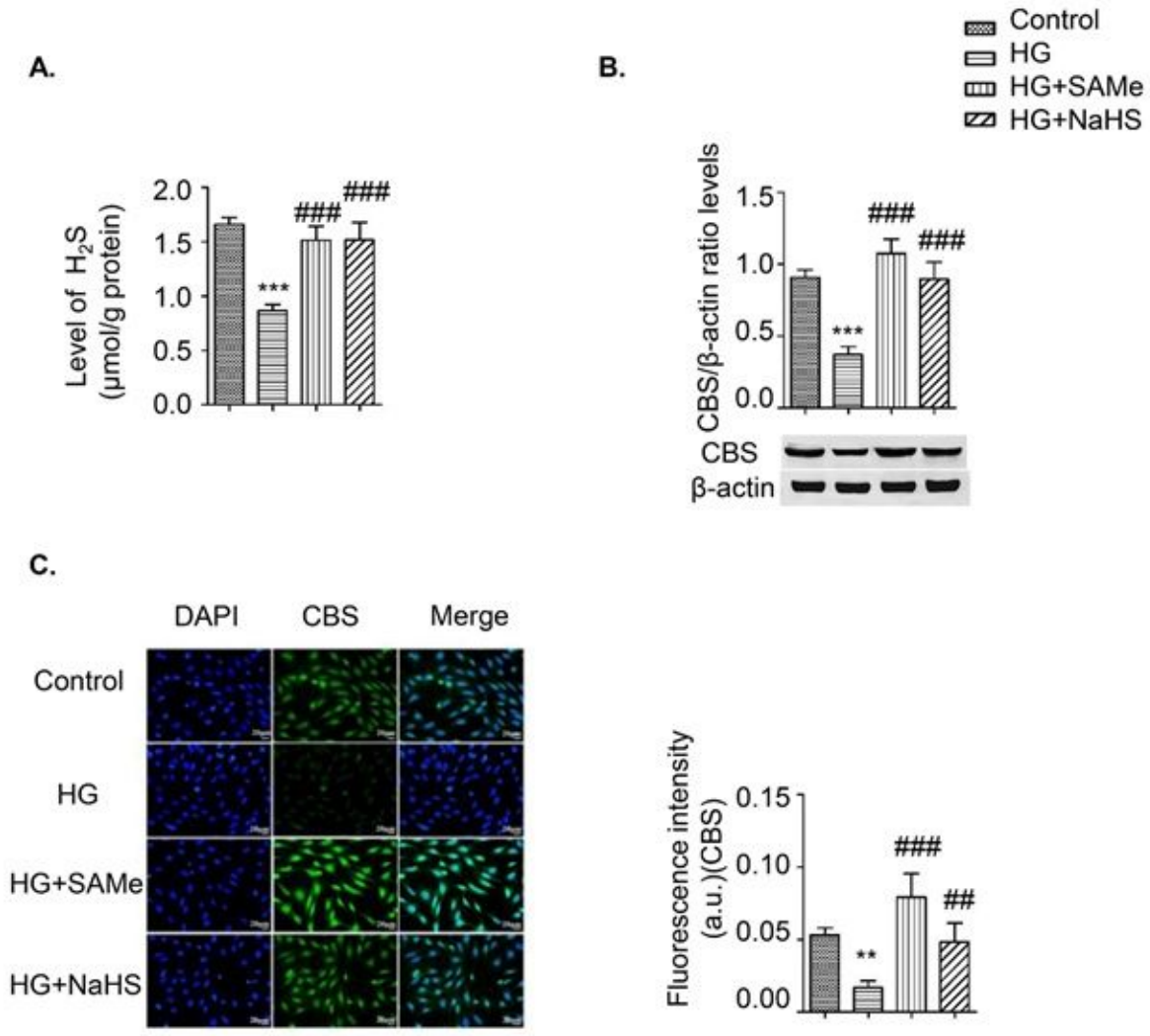

Figure 2

(A) Measurement of H2S level in HT-22 cells with NIR-NP probe. (B) Effects of H2S on the protein levels of CBS expression in HT-22 cells. Western blotting results showed that HG reduced CBS protein, which was restored by NaHS or SAMe. (C) The expression of CBS was analyzed by immunofluorescence. Scale bar = 20 μm. Data are presented as Mean ± SD (n = 3). **P < 0.01 and ***P < 0.001 vs control group; ##P < 0.01 and ###P< 0.001 vs HG group. Abbreviation: HG: high glucose.

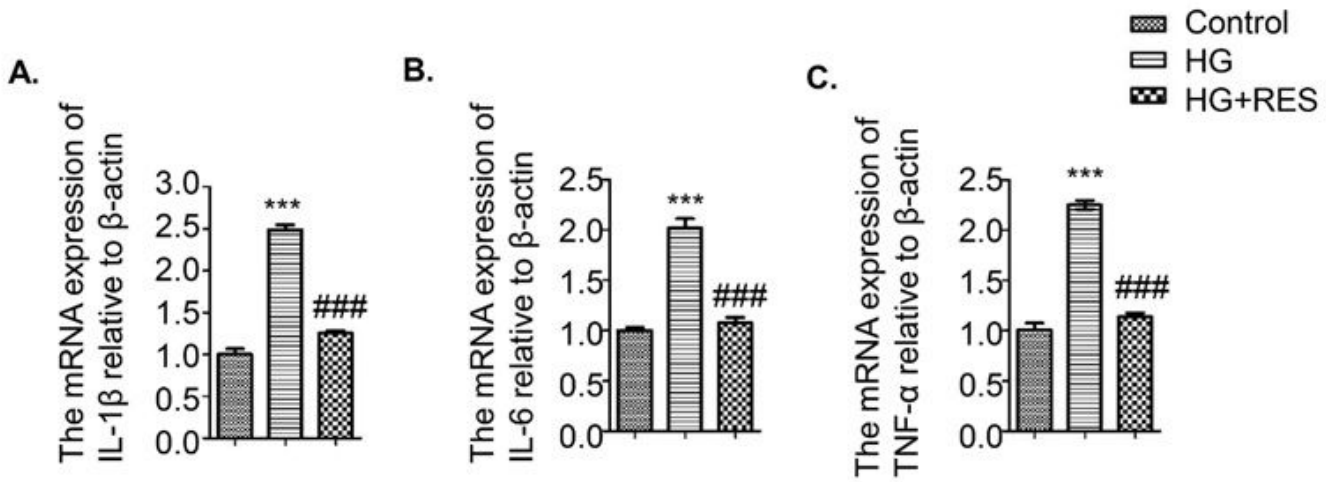

Figure 3

Effect of SIRT1 activation on HG-induced pro-inflammatory cytokine production. The regulated cytokines were (A) IL-1β, (B) IL-6 and (C) TNF-α. Data are presented as Mean ± SD (n = 3). ***P < 0.001 vs control group; ###P< 0.001 vs HG group. Abbreviations: HG: high glucose; RES: resveratrol.

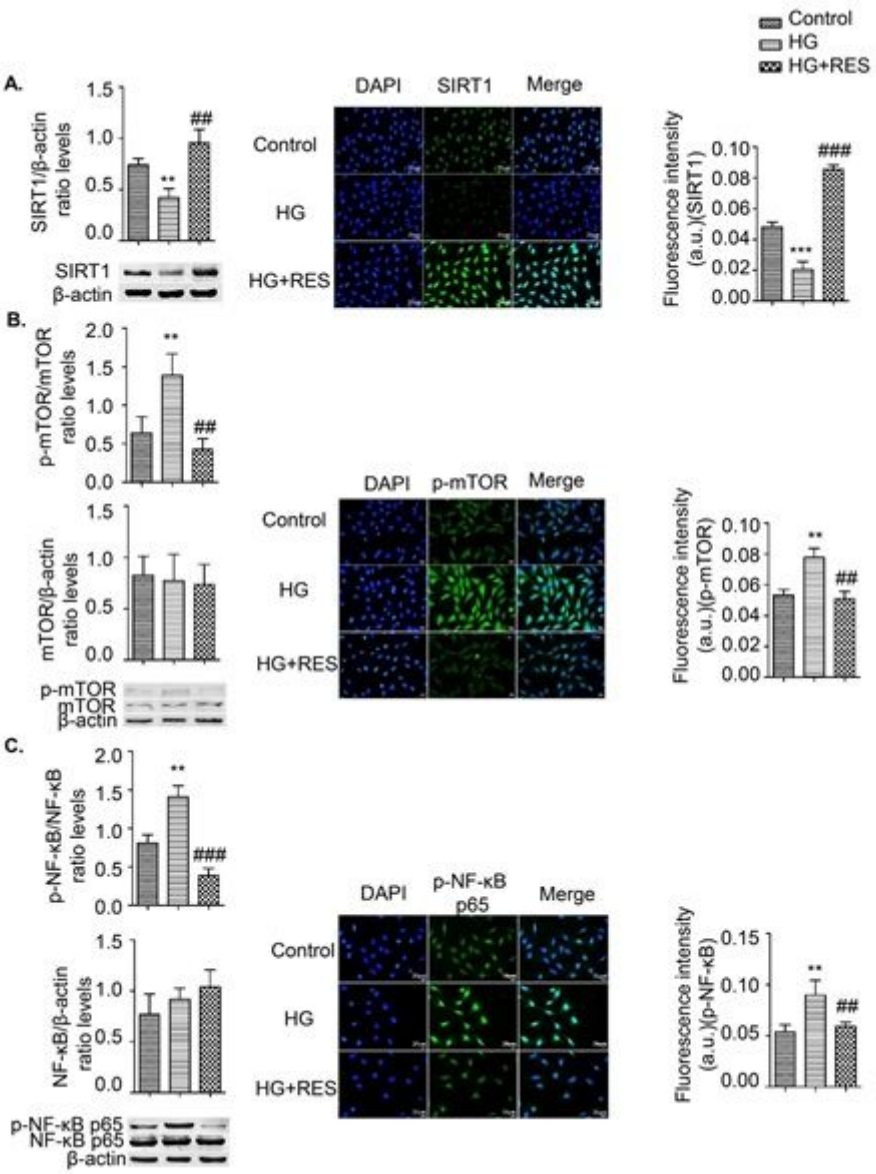

Figure 4

Effect of SIRT1 activation on the expression of mTOR and NF-κB p65 in HT-22 cells. (A) Western blotting and immunofluorescence results showed that HG reduced the expression of SIRT1, which was restored by resveratrol, a SIRT1 agonist. (B and C) Western blotting and immunofluorescence results showed that HG-induced phosphorylation of mTOR (B) or NF-κB (C) were restored by resveratrol. Total mTOR and NF-κB p65 proteins were not altered by either HG or resveratrol. Scale bar = 20 μm. Data are presented as Mean ± SD (n = 3). **P < 0.01 and ***P< 0.001 vs control group; ##P< 0.01 and ###P< 0.001 vs HG group. Abbreviations: HG: high glucose; RES: resveratrol.

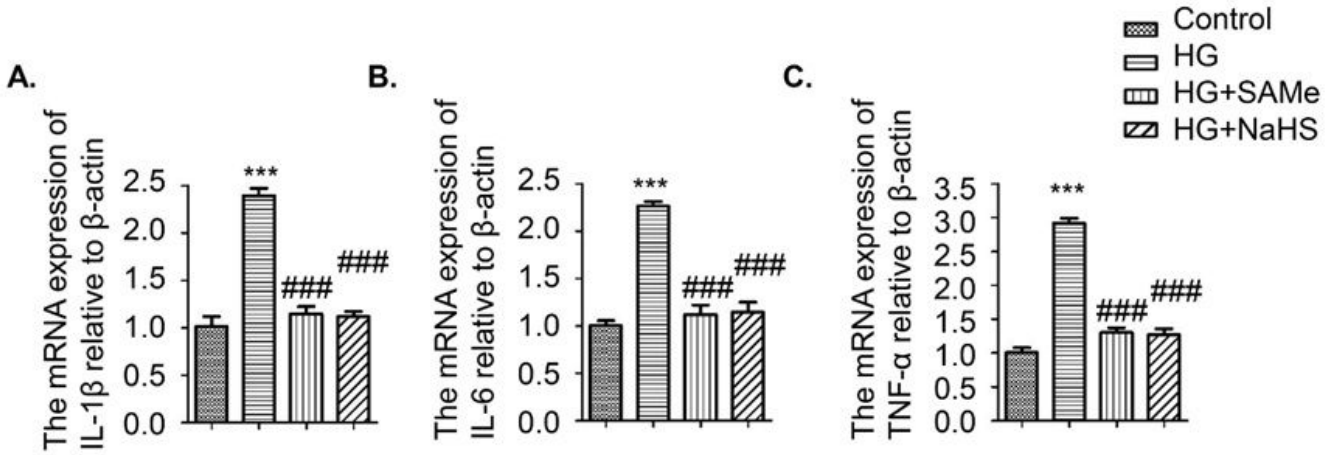

Figure 5

Effects of H2S on the production of pro-inflammatory cytokines in HT-22 cells. HG increased the mRNA expression of (A) IL-1β, (B) IL-6 and (C) TNF-α, which was inhibited by NaHS or SAMe treatment. Data are presented as Mean ± SD (n = 3). ***P < 0.001 vs control group; ###P< 0.001 vs HG group. Abbreviation: HG: high glucose.

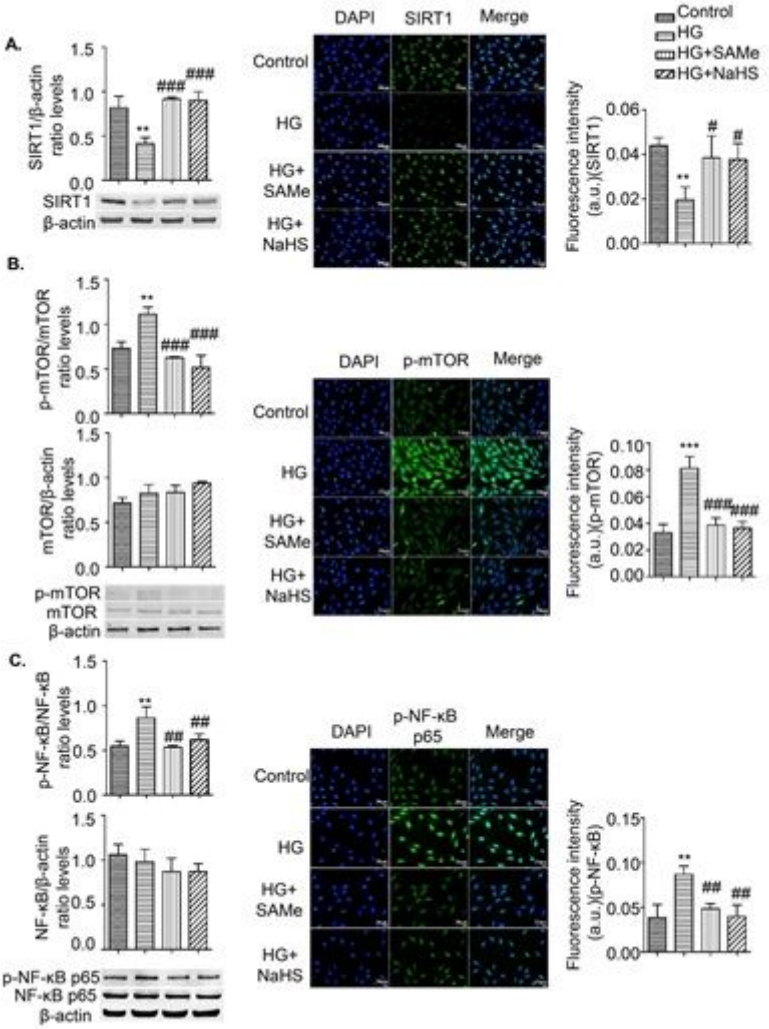

Figure 6

Effects of H2S on the expression of SIRT1, mTOR and NF-κB p65 in HT-22 cells. (A) Western blotting and immunofluorescence results showed that HG reduced the expression of SIRT1 protein, which was reversely by NaHS or SAMe. (B and C) Western blotting and immunofluorescence results showed that HG promoted the phosphorylation of mTOR (B) and NF-κB p65 (C), which was restored by NaHS or SAMe. Total mTOR and NF-κB p65 proteins were not altered by either HG, SAMe or NaHS. Scale bar = 20 μm. Data are presented as Mean ± SD (n = 3). **P < 0.01 and ***P< 0.001 vs control group; #P< 0.05, ##P< 0.01 and ###P< 0.001 vs HG group. Abbreviation: HG: high glucose.

Supplementary Files

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- SupplementaryMaterial.docx