Neuroendocrinology Letters Volume 44 No. 4 2023
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

The effect of hypoxia on Hydrogen Sulfide concentration of brain tissue in AD transgenic mice and its mechanism.

Xiumei Cheng[1], Meixia Ding[1], Churong Wei[2], Feng Xiao[2], Jiawan Liao[2], Mengjun Li[2], Zhiyong Xiao[2], Xiaoai Liu[3], Yougen Luo[1]

1 Yancheng Hemiplegia Rehabilitation Engineering Technology Research Center, Jiangsu Vocational College of Medicine, Yancheng, 224005, China.
2 The Research Center of Neurodegenerative Diseases and Aging, Medical College of Jinggangshan University, Ji'an, 343009, China.
3 Institute of Nursing, Guangdong Food and Drug Vocational College, Guangzhou, 510520, China.

Correspondence to: Dr. Yougen Luo
Yancheng Hemiplegia Rehabilitation Engineering Technology Research Center, Jiangsu Vocational College of Medicine, Yancheng, 224005, China
tel: +86 515 88588608, fax: +86 515 88159499, e-mail: lyougen@163.com

Submitted: 2023-01-20 Accepted: 2023-05-25 Published online: 2023-05-25

Key words: **Alzheimer's disease; Hypoxia; Hydrogen sulfide; Cerebral cortex; Cystathionine-β-synthase**

Neuroendocrinol Lett 2023; **44**(4):193–198 **PMID:** 37466058 NEL440423A02 • **www.nel.edu**

Abstract

OBJECTIVE: Research has shown that hydrogen sulfide (H_2S) plays a protective role in many diseases of the nervous system. The aim of this study is to investigate the effect of hypoxia on endogenous H_2S concentration in the cerebral cortex of Alzheimer's disease (AD) transgenic mice and its mechanism.
METHODS: AD transgenic mice were raised in closed boxes and pure nitrogen was introduced to reduce the oxygen concentration to 8%-10%, establishing an animal model of hypoxia. Oxygen partial pressure was measured with an oxygen meter. The expression of cystathionine-β-synthase (CBS) in cerebral cortex tissue was determined by Western blot, and H_2S concentration was measured by a modified methylene blue method.
RESULTS: (1) Hypoxia down-regul ated CBS expression in cerebral cortex tissue of AD transgenic mice ($p < 0.05$). (2) The concentration of H_2S in the cerebral cortex tissue of the hypoxic transgenic group was significantly lower than that of the Control group ($p < 0.01$). (3) Overexpression of CBS reversed the hypoxia-induced decrease of H_2S concentration in the cerebral cortex tissue of AD transgenic mice ($p < 0.01$).
CONCLUSIONS: Hypoxia decreased the concentration of endogenous H_2S in the cerebral cortex tissue of AD transgenic mice by down-regulating the expression of CBS.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by diffuse cerebral cortical atrophy, neurofibrillary tangles (NFT), and a substantial number of senile plaques (SP) between neurons (Ordóñez-Gutiérrez *et al.* 2021). Clinical symptoms of AD typically include a progressive decline in cognitive and memory function (Cass, 2017). As life expectancy continues to increase, the incidence of AD is rising rapidly. Since the pathogenesis of AD is still unclear and effective treatments are lacking, it is particularly important to study the pathogenesis of AD.

Hypoxia is a common pathological process that plays an important role in the pathogenesis of AD. Cerebral ischemia and stroke have been shown to significantly increase the incidence rate of AD (Alexander *et al.* 2022; Kazim *et al.* 2022). However, the pathogenesis of hypoxia-induced AD is far from being fully understood.

Hydrogen sulfide (H_2S) is considered a new gas signal molecule and plays a key role in the body (Rose *et al.* 2017). Studies have shown that H_2S can inhibit AD-like pathological changes. Cystathionide γ-lyase and cystathionide β-synthetase (CBS) in the body catalyze sulfur-containing amino acids to produce H_2S (Wang *et al.* 2022). Previous research from our research group has shown that H_2S inhibits neuronal apoptosis induced by hypoxia or OGD/R (Luo *et al.* 2013). Data also suggest that endogenous H_2S concentration, CBS activity (the main enzyme for H_2S synthesis), and S-adenosylmethionine (an agonist of CBS) content are decreased in AD patients (Dan *et al.* 2020; McCarty *et al.* 2019). Furthermore, Lan *et al.* demonstrated that hypoxia decreased the expression of CBS in cell experiments (Lan *et al.* 2011). However, it is still unclear whether hypoxia can reduce the concentration of H_2S in brain tissue by inhibiting the expression of CBS. This study aims to explore the effect of hypoxia on the concentration of H_2S in brain tissue and its underlying mechanism by replicating the hypoxia model in AD transgenic mice.

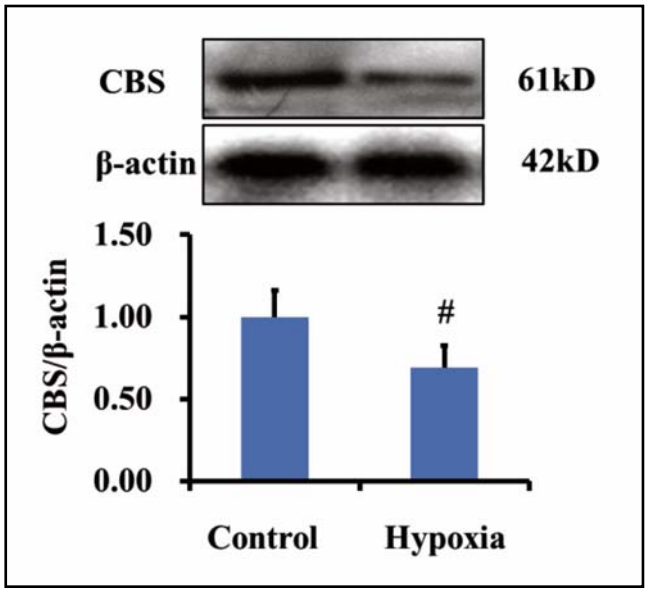

Fig. 1. The effect of hypoxia on CBS expression in cerebral cortex tissues of transgenic AD mice. Mice were maintained in a closed feeding box at 8%-10% oxygen partial pressure for 1 month and then euthanized by cervical dislocation. The cerebral cortex was taken out and protein was extracted. Protein concentration was measured by BCA method, and CBS protein expression was detected by Western blot analysis. Statistical analysis revealed significantly decreased CBS expression in the hypoxia-treated group compared to the Control group. #: $p < 0.01$, vs Control, n = 4.

MATERIALS AND METHODS

Main equipment and reagents

Protein electrophoresis and transfer system (Mini protein tetra and Mini tans-blot), Spectrophotometer (Beijing Purkinje General, TU-1810), Microplate reader (Bio-Rad, imark), Na2S (Shanghai Macklin, S888711, AR), $Zn(CH_3COO)_2$ (Shanghai Aladdin, Z110779, AR), $C_2HCl_3O_2$ (Shanghai Macklin, T818878, ACS), $NH_4Fe(SO_4)_2$ (Shanghai Aladdin, A112898, AR), NaOH (Shanghai Macklin, S817968, AR), N,N-Dimethyl-p-phenylenediamine (Shanghai Macklin, N835824, AR).

Animals and materials

AD transgenic mice (male, 10 months old) were acquired from Nanjing Biomedical Research Institute of Nanjing University and raised in the Clean Experimental Animal Research Center of Jinggangshan University. Anti-CBS and anti-GAPDH antibodies were obtained from ABCAM Company. HRP labeled secondary antibody, BCA protein quantitative kit, and ECL chemiluminescence kit were obtained from Shanghai Beyotime Biotechnology Co., Ltd. Chloral hydrate was purchased from Shenyang Chemical Reagent Factory. Overexpressed CBS lentivirus particles were constructed by Hanheng Biotechnology Co., Ltd.

Construction of hypoxia model

AD transgenic mice were placed in a closed feeding box with food and water. Pure nitrogen was introduced into the air inlet to create a hypoxic environment. Throughout the experiment, the oxygen partial pressure in the feeding box was measured with an oxygen meter and manually controlled to maintain the oxygen content at 8%-10%. To avoid the influence of other variables, an appropriate amount of calcium chloride and sodium lime was placed in the feeding box to absorb the water vapor and carbon dioxide exhaled by the AD transgenic mice. The AD transgenic mice were treated in the hypoxic environment for 10 hours per day and maintained for a period of 1 month.

Immunoblotting

After treatment of AD transgenic mice, the cervical spine was dislocated and euthanized. The brain was

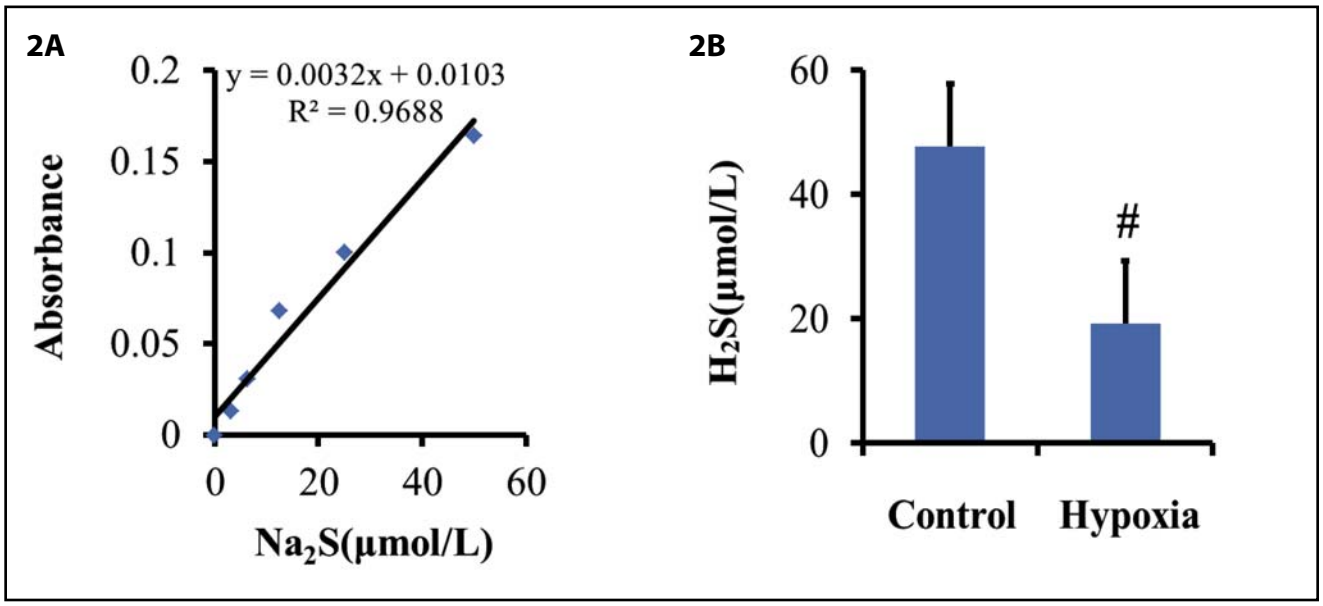

Fig. 2. The effect of hypoxia on the concentration of hydrogen sulfide in cerebral cortex. The absorbance values of Na_2S solution with concentration of 3.125, 6.25, 12.5, 25 and 50μmol/L at 665 nm were determined by spectrophotometer. The concentration of Na_2S was set as X-axis, the absorbance values were set as Y-axis, and the standard curves were made (2A). After 1 month of hypoxia treatment in transgenic AD mice, cerebral cortex was taken out and homogenized. Modified methylene blue method was used to determine H_2S concentration (2B). #: $p < 0.01$, vs Control, n = 3.

cut off and the cortex was separated. 100 mg of cortical tissue was added to 10 times the volume of protein lysis solution and homogenized for 20 seconds. The supernatant was centrifuged at 4°C and 12000 rpm for 10 minutes. The supernatant was then stored at -80°C, and the protein concentration was measured by the BCA method. Then, an immunoblotting experiment was performed according to the previous method (Bai *et al.* 2015), including the following steps in order: sample loading, electrophoresis, membrane transfer, blot blocking, primary antibody incubation, secondary antibody incubation, and color development. The electrophoresis conditions were as follows: 5% concentrated gel at 80V for 30-60 minutes, 10% separated gel at 100V for 60-120 minutes. The specific time was adjusted according to the pre-stained marker instructions. The conditions of membrane transfer were as follows: wet transfer, 275mA for 90 minutes, the time was adjusted according to the molecular weight of the target protein. The primary antibody was diluted to 1:500-1:1000 and incubated overnight at 4°C. The secondary antibody was diluted to 1:2500 and incubated for 1h at room temperature, followed by ECL color development.

Determination of H_2S concentration by modified methylene blue method

After treatment with AD transgenic mice, they were dislocated and euthanized. The brain was isolated and placed in ice PBS solution. The cortical tissue was rapidly isolated and stored at -80°C for the detection of H_2S concentration.

The optical density (OD) values of 3.125, 6.25, 12.5, 25, 50μM Na_2S solution was measured with spectrophotometry at 665 nm. The Na_2S concentrations and OD values were plotted on the X-axis and Y-axis, respectively, to construct standard curves. These curves were then used to calculate the concentration of H_2S.

$Zn(CH_3COO)_2$ was prepared as a 5% solution, and 100 μL of this solution was added to 100 μL of cerebral cortex tissue samples, then 100 μL of 5 mol/L NaOH was added after sufficient shaking, followed by centrifugation at 15000 g for 10 minutes at low temperature (4°C). The supernatant was aspirated by vacuum pump and 500 μL of deionized water was added and washed. The supernatant was discarded after centrifugation at 15000 g for 10 minutes. Precipitation was added by 100 μL of 5% $Zn(CH_3COO)_2$, 0.2% N, N-Dimethyl-p-phenylenediamine and 20% $C_2HCl_3O_2$, respectively, then centrifuge at 15000 g for 5 minutes. We sucked out 200 μL supernatant and added 10μL of 10% $NH_4Fe(SO_4)_2$ into the supernatant. The mixed liquid was fully oscillated and stood for 5-15 minutes, then took 200 μL solution to add to 96 well plate. Its absorbance was measured at 665 nm according to the spectral scanning results. H_2S concentration was calculated in the solution according to the standard curve (Zheng *et al.* 2012).

Cortical injection with Lentivirus particles

According to the literature (Kimura *et al.* 2010), AD transgenic mice were anesthetized with intraperitoneal administration of 6% chloral hydrate, and 2 μL of CBS overexpression lentivirus particles (injection titer 10^6 TU) were injected into the cortex using the following location coordinates: AP 1.5 mm, L 2.0 mm, and H 0.8-1.0 mm. These coordinates correspond to 1.5 mm

posterior to the anterior fontanelle, 2.0 mm lateral to the right side, and 0.8-1.0 mm inferior to the subcortex. A small hole was made in the skull at the coordinate point, and lentivirus particles were injected at a rate of 1 μL/min. After each 1 μL injection of lentiviral particles, the needle was left in place for 1 minute, and after the injection was completed, the needle was left in place for 15 minutes before being slowly withdrawn.

Data processing

The experimental data were expressed by Mean ± SD and analyzed using SPSS 20.0 statistical software. The comparison between two groups was performed by t-test, while the differences among groups were determined using one-way ANOVA. Comparison among them was performed by LSD-t-test. In all experiments, $p < 0.05$ was considered statistically significant.

RESULTS

Effect of hypoxia on CBS expression in AD transgenic cerebral cortex

After 1 month of hypoxia treatment, AD transgenic mice were anesthetized with chloral hydrate. Cerebral cortex was extracted and homogenized with RIPA lysate to extract total protein. Protein concentration was measured by the BCA method. CBS protein expression was detected by Western blot analysis. The results showed that after 1 month of hypoxia treatment, the expression of CBS in the cerebral cortex of AD transgenic mice decreased to (69.23 ± 13.36)% of the Control group ($p < 0.01$, n = 4, Figure 1).

Effect of hypoxia on H_2S concentration in cerebral cortex

The absorbance values obtained from the spectrophotometry were used to construct a standard curve (Figure 2A). The linear equation derived was y = 0.0032x + 0.0103 with an R^2 value of 0.9688. Based on this equation, H_2S concentrations were calculated. Our results showed that hypoxia led to a decrease in the concentration of H_2S in cerebral cortical tissue, with the level decreasing from 47.81 ± 5.99 μmol/L in the Control group to 19.30 ± 3.66 μmol/L in the Hypoxia group ($p < 0.01$, n = 3, Figure 2B).

Role of CBS in hypoxia-induced decrease in H_2S concentration in AD transgenic mice brain tissue

To clarify whether CBS mediated the hypoxia-induced decrease in H_2S concentration of cerebral cortex tissue, after 48h of cortical injection of normal saline, empty virus particles (EV), or CBS overexpression lentivirus particles (CBS^{OE}) in mice, AD transgenic mice were exposed to 1 month of hypoxia. Blank control (Control) mice were injected with normal saline and maintained in a normoxic environment for one month. CBS expression and H_2S concentration in cerebral cortex tissue were assessed using the aforementioned methods.

Immunoblotting analysis revealed a significant decrease in CBS expression in the Hypoxia group compared to the Control group ($p < 0.01$, n = 5, Figure 3A). However, CBS expression in the hypoxia + CBS^{OE} group was significantly higher than that in the Hypoxia group ($p < 0.01$, n = 5, Figure 3A). These results indicated that cortical injection of CBS-overexpressing lentiviral particles was able to prevent the hypoxia-induced reduction of CBS expression.

The H_2S concentration was measured by the modified methylene blue method, and it was found that the H_2S concentration of the hypoxia group was lower than that of Control group in the cerebral cortex tissue of transgenic mice ($p < 0.01$, n = 5, Figure 3B), while the H_2S concentration of the hypoxia + CBS^{OE} group was significantly higher than that of the hypoxia group ($p < 0.01$, n = 5, Figure 3B).

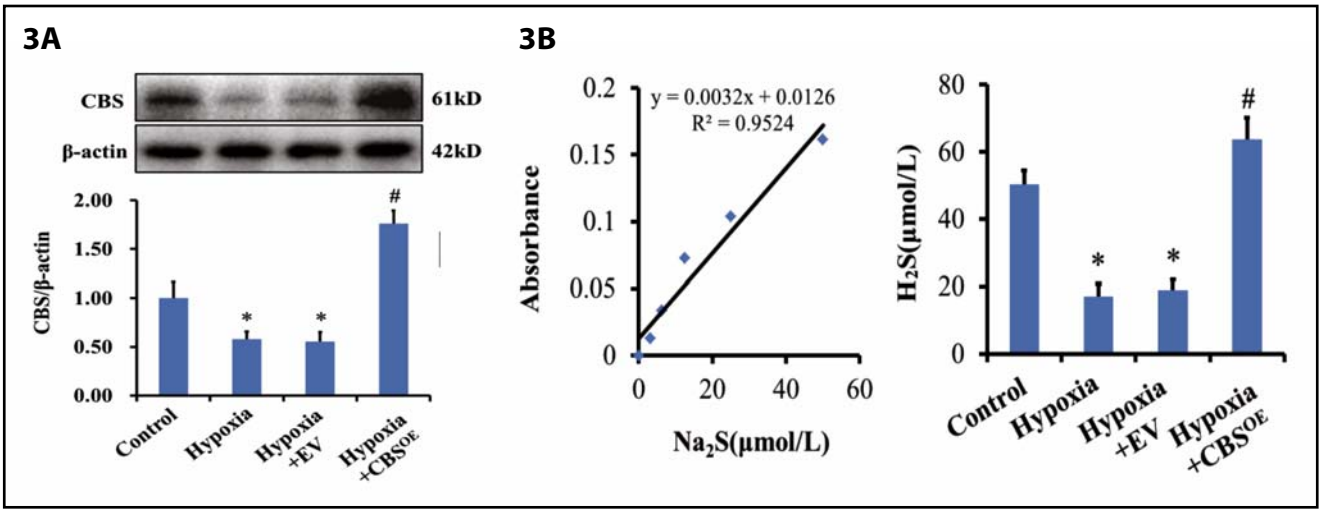

Fig. 3. The role of CBS in the decrease of H_2S concentration induced by hypoxia in AD transgenic mice brain. A: After 48h of cortical injection of normal saline, EV, or CBS^{OE} in mice, AD transgenic mice were exposed to 1 month of hypoxia. The expression of CBS protein in AD transgenic mice was detected by Western blot. B: The concentration of H_2S in cerebral cortex of AD transgenic mice was detected by modified methylene blue method. *: $p < 0.01$, vs Control; #: $p < 0.01$, vs Hypoxia, n = 5.

DISCUSSION

H_2S, as the third gas signal molecule, is known for its wide range of cytoprotective effects. It exhibits anti-oxidant and anti-apoptotic properties (Kimura *et al.* 2010; Heruye *et al.* 2022; Zhu *et al.* 2022). It could facilitate long-term potentiation (LTP) and reduce the damage of spatial learning and memory in animals by increasing the excitability of hippocampal neurons (Liu *et al.* 2017). Moreover, H_2S can inhibit learning and memory impairment and neuroinflammatory response induced by lipopolysaccharide (Kshirsagar *et al.* 2021; Mai *et al.* 2018). Interestingly, studies have shown that H_2S can also inhibit the expression of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) mRNA and protein through the PI3K/Akt signaling pathway, thereby significantly reducing $A\beta_{42}$ production in PC12 cells (Zhang *et al.* 2011). These findings suggest that H_2S may inhibit the occurrence and development of AD-like lesions.

Hypoxia has been shown to induce AD-like pathological changes. Zhang *et al.* demonstrated that BACE1 expression was up-regulated and Aβ production was increased under hypoxic conditions (Zhang *et al.* 2015). Additionally, antioxidants have been found to inhibit BACE1 expression in AD mice and reduce the hypoxic area of brain tissue, thereby suppressing AD-like pathological changes (Javier *et al.* 2018). Moreover, hypoxia has been shown to significantly increase the phosphorylation levels of tau protein at multiple sites such as Ser198/199/202. This is achieved through glycogen synthase kinase-3β (GSK-3β) activation and the inhibition of protein phosphatase 2A (PP-2A) activity (Zhang *et al.* 2014).

Hypoxia might be involved in nerve cell injury by affecting the synthesis of endogenous H_2S. Chemical hypoxia treatment with 600 mmol/L cobalt chloride decreased the expression of CBS, which is an essential enzyme for endogenous H_2S synthesis. As a result, nerve cells were significantly damaged (Lan *et al.* 2011). Similarly, Liu *et al.* observed reduced levels of CBS mRNA and protein in ischemic and hypoxic brain tissue, resulting in significant hypoxic-ischemic injury in brain tissue of newborn mice (Liu *et al.* 2017). Despite these findings, there have been conflicting reports on the effects of hypoxia on CBS expression in brain tissue. For example, Shi *et al.* found that CBS had higher expression in cerebral white matter areas with hypoxia-ischemia from 12 hours to 7 days in neonatal SD rats, suggesting that CBS may play a neuroprotective role against hypoxic injury (Shi *et al.* 2014). Mishra *et al.* exposed rats to low pressure and oxygen for 1-7 days and found increased CBS expression but decreased H_2S levels in the brain. Based on their analysis, the increased CBS expression in the brain may be an adaptive regulation of the body to the low-pressure hypoxic environment (Mishra *et al.* 2020).

To investigate the effect of long-term hypoxia on the concentration of H_2S in brain tissue and its mechanism, AD transgenic mice were placed in a closed feeding box and exposed to nitrogen to create a classic animal hypoxia model. Calcium chloride and sodium lime were also added to eliminate the effects of water vapor and carbon dioxide exhaled by the mice. To observe the long-term effects of hypoxia, the hypoxic treatment was extended to one month. The results showed that hypoxia down-regulated the expression of CBS and reduced the concentration of H_2S in the cerebral cortex of AD transgenic mice. Overexpression of CBS was found to inhibit the low expression of CBS induced by hypoxia and increase the concentration of H_2S. These results suggest that hypoxia inhibits the production of endogenous H_2S by down-regulating the expression of CBS, which promotes the pathogenesis of AD. Our studies provide a novel target for the prevention and treatment of AD caused by hypoxia-ischemia. However, the detailed mechanisms by which hypoxia inhibits CBS expression remain unclear and require further investigation.

ACKNOWLEDGEMENTS

This work was supported by the NSFC (National Natural Science Foundation of China) [31660271], the science and technology support project of Jiangxi Provincial Department of Education [No.GJJ160731, GJJ180580, GJJ190544, GJJ201027, GJJ211014, GJJ2201615], Guangdong Medical Science and technology research fund project [No. A2020164], and Jiangsu Vocational College of Medicine research fund project [20204102].

DECLARATION OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 Alexander C, Li T, Hattori Y, Chiu D, Frost GR, Jonas L, et al. (2022). Hypoxia inducible factor-1α binds and activates γ-secretase for Aβ production under hypoxia and cerebral hypoperfusion. Mol Psychiatry. **27**(10): 4264–4273.

2 Bai S, Mao M, Tian L, Yu Y, Zeng J, Ouyang K, et al. (2015). Calcium sensing receptor mediated the excessive generation of β-amyloid peptide induced by hypoxia in vivo and in vitro. Biochem Biophys Res Commun. **459**(4): 568–573.

3 Cass SP (2017). Alzheimer's disease and exercise: A literature review. Curr Sports Med Rep. **16**(1): 19–22.

4 Dan ZF, Wu DD, Zhao D, Sun JJ (2020). The level of homocysteine and hydrogen sulfide in senile dementia and its clinical significance. Sichuan Journal of Anatomy. **28**(3): 137–138.

5 Heruye SH, Mbye YF, Ohia SE, Opere CA (2022). Protective action of hydrogen sulfide-releasing compounds against oxidative stress-induced cataract formation in cultured bovine lenses. Curr Eye Res. **47**(2): 239–245.

6 Javier FR, Francisco JSB, Juan RP, Frank ML, Lydia GL, Mario DP, et al. (2018). Sex-dependent co-occurrence of hypoxia and β-amyloid plaques in hippocampus and entorhinal cortex is reversed by long-term treatment with ubiquinol and ascorbic acid in the 3 × Tg-AD mouse model of Alzheimer's disease. Mol Cell Neurosci. **92**: 67–81.

7 Kazim SF, Sharma A, Saroja SR, Seo JH, Larson CS, Ramakrishnan A, et al. (2022). Chronic intermittent hypoxia enhances pathological tau seeding, propagation, and accumulation and exacerbates Alzheimer-like memory and synaptic plasticity deficits and molecular signatures. Biol Psychiatry. **91**(4): 346–358.

8 Kimura Y, Goto Y, Kimura H (2010). Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. Antioxid Redox Signal. **12**(1): 1–13.

9 Kshirsagar V, Thingore C, Gursahani M, Gawali N, Juvekar A (2021). Hydrogen sulfide ameliorates lipopolysaccharide-induced memory impairment in mice by reducing apoptosis, oxidative, and inflammatory effects. Neurotox Res. **39**(4): 1310–1322.

10 Lan A, Liao X, Mo L, Yang C, Yang Z, Wang X, et al. (2011). Hydrogen sulfide protects against chemical hypoxia-induced injury by inhibiting ROS-activated ERK1/2 and p38MAPK signaling pathways in PC12 cells. PLoS One. **6**(10): e25921.

11 Liu HX, Yu LM, Huang J, Zhang LX, Yue Z, Li QZ (2017). Effect of H_2S system on space learning and memory ability in PTSD model rat and on the frequency of spontaneous discharges of hippocampus neurons. Chinese Pharmacological Bulletin. **33**(2): 206–211.

12 Liu S, Xin D, Wang L, Zhang T, Bai X, Li T, et al. (2017). Therapeutic effects of L-Cysteine in newborn mice subjected to hypoxia-ischemia brain injury via the CBS/H_2S system: Role of oxidative stress and endoplasmic reticulum stress. Redox Biol. **13**(14): 528–540.

13 Luo Y, Yang X, Zhao S, Wei C, Yin Y, Liu T, et al. (2013). Hydrogen sulfide prevents OGD/R-induced apoptosis via improving mitochondrial dysfunction and suppressing an ROS-mediated caspase-3 pathway in cortical neurons. Neurochem Int. **63**(8): 826–31.

14 Mai MA, Ola SMA, Laila AR, Badawi AM, Nadia AE (2018). Regulation of mirna-124, nuclear factor-kappa b and β-catenin expression in response to novel therapeutic protocol in LPS induced alzheimer's disease in rats. Research in Neuroscience. 2018, **7**(1): 14–30.

15 McCarty MF, O'Keefe JH, DiNicolantonio JJ (2019). A diet rich in taurine, cysteine, folate, B12 and betaine may lessen risk for Alzheimer's disease by boosting brain synthesis of hydrogen sulfide. Med Hypotheses. **132**: 109356.

16 Mishra S, Kumar G, Chhabra A, Sethy NK, Jain N, Meena RN, et al. (2020). Cysteine becomes conditionally essential during hypobaric hypoxia and regulates adaptive neuro-physiological responses through CBS/H_2S pathway. Biochim Biophys Acta Mol Basis Dis. **1866**(7): 165769.

17 Ordóñez-Gutiérrez L, Fábrias G, Casas J, Wandosell F (2021). Diets with higher ω-6/-3 ratios show differences in ceramides and fatty acid levels accompanied by increased amyloid-beta in the brains of male APP/PS1 transgenic mice. Int J Mol Sci. **22**(20): 10907.

18 Rose P, Moore PK, Zhu YZ (2017). H_2S biosynthesis and catabolism: New insights from molecular studies. Cell Mol Life Sci. **74**(8): 1391–1412.

19 Shi JP, Tang CH, Shang Y, Cao YL, Gao J, Yang WH (2014). Expression of hydrogen sulphide and cystathionine -beta-synthase in immature rats with periventricular leukomalacia. Journal of Xinxiang Medical University. **31**(1): 15–18.

20 Wang S, Ding Y, Jiang W (2022). CSE/H_2S ameliorates colitis in mice via protection of enteric glial cells and inhibition of the RhoA/ROCK pathway. Front Immunol. **13**: 966881.

21 Zhang CE, Yang X, Li L, Sui X, Tian Q, Wei W, et al. (2014). hypoxia-induced tau phosphorylation and memory deficit in rats. Neurodegener Dis. **14**(3): 107–116.

22 Zhang H, Gao Y, Zhao F, Dai Z, Meng T, Tu S, et al. (2011). Hydrogen sulfide reduces mRNA and protein levels of β-site amyloid precursor protein cleaving enzyme 1 in PC12 cells. Neurochem Int. **58**(2): 169–175.

23 Zhang XW, Cui J, Li L (2015). Effects of hypoxia on the expressions of miR-124 and BACE1 protein in SH-SY5Y cells. Basic&Clinical Medicine. **35**(4): 470–474.

24 Zheng Y, Liao F, Du JB, Tang CS, Xu GH, Geng B (2012). Modified methylene blue method for measurement of hydrogen sulfide level in plasma. Acta Physiologica Sinica. **64**(6): 681–686.

25 Zhu S, Li X, Dang B, Wu F, Gou K, Wang C, et al. (2022). Hydrogen sulfide protects retina from blue light-induced photodamage and degeneration via inhibiting ROS-mediated ER stress-CHOP apoptosis signal. Redox Rep. **27**(1): 100–110.