Eurasia Journal of Science and Technology
ISSN: 2663-1016
DOI: 10.61784/ejst3017

OPN PROMOTES HYPERGLYCEMIA WITH CEREBRAL HEMORRHAGE AND NEUROINFLAMMATORY INJURY BY INDUCING MICROGLIA AND MACROPHAGE INFLAMMATION

RuiMing Fan[1], Wei Bu[2], Yu Wei[2], LiLi Wu[2], YuanWen Liu[2], Lei Gong[3*]
[1]*Cerebrovascular Department, Affiliated Hospital of Zunyi Medical University, Zunyi 563000, Guizhou, China.*
[2]*Pharmacy Department, Affiliated Hospital of Zunyi Medical University, Zunyi 563000, Guizhou, China.*
[3]*Guizhou Engineering Research Center for Translational Medicine, Zunyi Medical University, Zunyi 563000, Guizhou, China.*
Corresponding Author: Lei Gong, Email: gonglei28@126.com

Abstract: Objective: To explore the role of OPN in hyperglycemia with cerebral hemorrhage and neuroinflammatory injury and its preliminary mechanism. Methods: BV2 cells/RAW264.7 macrophages were stimulated with 30 ng/mL OPN for 24 hours in advance, and then cultured with 33 mmol/L high glucose medium for 24 hours. The expression of IL-1 β and iNOS in RAW264.7 cells was detected by RT-PCR, and the expression of TNF - α in BV2 cells was detected by Western blotting. The hyperglycemia model of rats after ICH (ICH+HG) was constructed, and the intervention was performed with anti OPN monoclonal antibody (0.4 mg/kg) and intraperitoneal injection of endoplasmic reticulum stress inhibitor (TUDC) (200 mg/kg). After administration, the neurological function score and HE staining of intracerebral hemorrhage tissues were performed, and the secretion levels of iNOS and TNF - α in intracerebral hemorrhage tissues were detected by ELISA. Results: In RAW264.7 cells, high glucose culture significantly increased the expression levels of iNOS and IL-1 β. Compared with high glucose group, OPN further increased the expression levels of iNOS and IL-1 β. In BV2 cells, high glucose culture significantly increased the expression of IL-1 β and TNF - α. Compared with high glucose group, OPN further increased the expression of IL-1 β and TNF - α. The neurological function score of rats in ICH+HG group increased significantly. Compared with the model group, the neurological function scores of rats in OPN monoclonal antibody group and TUDC group decreased. Conclusion: OPN can promote hyperglycemia associated with cerebral hemorrhage by inducing microglia and macrophage inflammation.
Keywords: Bone bridging proteins; Hyperglycemia; Cerebral hemorrhage; Neuroinflammation

1 INTRODUCTION

Hyperglycemia combined with cerebral hemorrhage is a condition in which cerebral hemorrhage occurs on top of hyperglycemia. Hyperglycemia is a condition in which blood glucose levels are above the normal range and is common in people with diabetes[1]. Cerebral hemorrhage is bleeding caused by the rupture of a blood vessel in the brain, which seriously affects the function of the brain[2]. High blood sugar causes damage to the walls of blood vessels. High blood sugar damages the walls of blood vessels, making them fragile and prone to rupture, increasing the risk of brain hemorrhage. In addition, cerebral hemorrhage causes large amounts of blood to enter the brain tissue, resulting in increased pressure in the brain and damage to nerve cells and brain function. Hyperglycemia also exacerbates the inflammatory response to brain hemorrhage, increasing the degree and extent of brain damage[3, 4]. Hyperglycemia and cerebral hemorrhage are two separate diseases with some conflicting treatment strategies and drug choices. Hyperglycemia and cerebral hemorrhage are two separate disorders with some conflicting treatment strategies and drug choices; for example, cerebral hemorrhage requires inhibition of coagulation to control bleeding, whereas patients with hyperglycemia may require procoagulants to prevent vasculopathy. This therapeutic conflict confuses physicians in formulating a treatment plan[5, 6]. The molecular mechanism of cerebral hemorrhage in hyperglycemia is of great significance. Therefore, exploring the molecular mechanism of the pathogenesis of hyperglycemia combined with cerebral hemorrhage is of great significance for developing clinical treatment of hyperglycemia combined with cerebral hemorrhage.

Neuroinflammation has an important impact on hyperglycemia combined with cerebral hemorrhage. Neuroinflammatory response will further exacerbate the inflammatory response caused by cerebral hemorrhage, leading to a greater degree of cerebral injury and increased nerve cell death and dysfunction. At the same time, neuroinflammation activates inflammatory mediators, further damaging the cerebral blood vessel walls and increasing the risk of hemorrhage[7]. Microglia are the major immune cells in the central nervous system, and they play an important role in the neuroinflammatory process. When nerve tissue is damaged or infected, microglia are activated and release inflammatory mediators such as cytokines and chemokines. These inflammatory mediators can elicit an inflammatory response in the brain, attracting other immune cells to the lesion and exacerbating the degree of neuroinflammation[8, 9]. In addition, macrophages are activated and migrate to the site of injury, infection or inflammatory stimuli. In addition, when nerve tissue is stimulated by injury, infection or inflammation, macrophages are activated and migrate to the site of the lesion. Activated macrophages can release a variety of inflammatory

© By the Author(s) 2024, under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0).

mediators, such as cytokines, chemokines, and oxygen free radicals. Excessive activation of macrophages may lead to uncontrolled inflammatory response and damage to neural tissues. Excessive release of inflammatory mediators may lead to exacerbation of inflammatory injury and even trigger cell death and tissue damage[10, 11]. The results of this study are summarized in the following table. Thus, microglia and macrophage hyperactivation may be involved in the pathogenesis of neuroinflammatory injury in hyperglycemia combined with cerebral hemorrhage.

Osteopontin (OPN) is a glycoprotein, which is widely distributed in the body, participates in a variety of biological processes, and has a variety of functions, including cell adhesion, migration, proliferation, inflammation regulation, etc[12]. OPN also plays an important role in the regulation of inflammation. It can regulate the activation and migration of immune cells and promote the occurrence and development of inflammatory reaction. In the process of inflammation, OPN can attract inflammatory cells into the focus and participate in the production and release of inflammatory mediators, thus affecting the degree and duration of inflammatory reaction[13, 14]. This study will explore the effect of OPN on the activation of microglia and macrophages to reveal its potential function in hyperglycemia with intracerebral hemorrhage, and provide a potential molecular target for clinical treatment of hyperglycemia with intracerebral hemorrhage.

2 MATERIALS AND METHODS

2.1 Cells and Treatment

Mouse microglia BV2 cell line (iCell-m011, Subekang) and mouse RAW264.7 macrophages (iCell-m047, Subekang) were purchased from Subekang. Both cells were cultured in complete DMEM medium (KGM12800, Keji Biology) under 5% CO2 and 37 ℃. In order to establish an in vitro high glucose induction model, BV2 cells/RAW264.7 macrophages were cultured for 24 hours in 33 mmol/L high glucose medium according to conventional methods. In order to explore the effect of OPN on the activity of high glucose stimulated BV2 cells/RAW264.7 macrophages, 30 ng/mL OPN was used to stimulate BV2 cells/RAW264.7 macrophages for 24 hours, and then 33 mmol/L high glucose medium was used to culture them for 24 hours according to the conventional method.

Real time fluorescent quantitative PCR (RT-PCR): use the pipette gun to suck out the cell culture medium in the culture dish, add Trizon lysate into the culture dish, blow with the pipette gun, make the adherent cell suspension fully contact with the lysate, collect all cell suspensions to extract RNA. The concentration and purity of RNA were determined by ultraviolet visible spectrophotometer (OD260/OD280). RNA was synthesized into cDNA through RNA reverse transcription kit. Fluorescent quantitative PCR was performed by using fluorescent PCR instrument. The reaction system is as follows: 2 × SYBR Green PCR Master Mix 10 μ l, cDNA 1 μ l, upstream primer 0.4 μ l, downstream primer 0.4 μ l RNase Free ddH20 8.2 μl。 The reaction steps are as follows: pre denaturation at 95 ℃ for 10 min; Denaturation 95 ℃, 10 s; Annealing at 58 ℃ for 30s; Elongation 72 ℃, 30s; 40 cycles. The sequence is shown in the following table. β - actin was used as the internal reference, and the relative expression of iNOS, IL-1 β, TNF - α was calculated according to the 2 -△△Ct method. See Table 1 for primer sequence.

Table 1 Primer Sequence in RTPCR Experiment

primer name	Primer sequence (5'-3')	Product length (bp)	Annealing temperature (℃)
β-actin F	AGGGAAATCGTGCGTGAC	192	58.0
β-actin R	CATACCCAAGAAGGAAGGCT		
iNOS F	GATGTGCTGCCTCTGGTCTTG	108	60.8
iNOS R	CCACTCGTACTTGGGATGCTC		
TNF-α F	CAGGCGGTGCCTATGTCTC	89	60.3
TNF-α R	CGATCACCCCGAAGTTCAGTAG		
IL-1β F	GAAATGCCACCTTTTGACAGTG	116	58.93
IL-1β R	TGGATGCTCTCATCAGGACAG		

2.2 Western Blot Detection

Discard the cell culture medium in the culture dish, add 100 μ L cell lysate into each hole, and place it on ice for 20 min. The pipette gun sucks into the marked EP pipe. Centrifuge with a 12000 r/min high-speed centrifuge for 10 min, discard the precipitation, take the supernatant and transfer it to a new EP tube (BCA determination), and store the total protein at - 80 ℃. Determine the protein concentration according to BCA kit, denature the protein, apply the sample to

SDS-PAGE for 1.5 h, and then apply 300 mA constant flow membrane for 1.5 h. Incubate the primary anti TNF - α (1:1000bs-0078r, Bios, China) with PVDF membrane, stay overnight at 4 ℃, incubate the secondary anti TNF - α with PVDF membrane at room temperature the next day for 2 hours, wash the membrane, wet the PVDF membrane with luminescent solution, and place it in the sample storage area of the ultra-high sensitivity chemiluminescence imaging system to run program development imaging.

Animal model construction and grouping administration: according to the method before John H Zhang's laboratory[15]. ICH+HG model was prepared by collagenase and 50% dextrose method. Collagenase VII was injected into the left cerebral basal ganglia (coordinates: 0.2 mm front, 6.0 mm belly, 3.0 mm outside), 0.075 units of collagenase was dissolved in 0.5 μ l normal saline, 0.25 μ l/min was injected for 2 min, and the rat ICH model was replicated. Three hours after ICH, 50% dextrose was injected intraperitoneally at the rate of 4g/kg to create a hyperglycemic model after ICH (ICH+HG). The ICH+HG model was made by intraperitoneal injection of 10 ml of 50% dextrose injection after establishing the collagenase basal ganglia injection method in rats with intracerebral hemorrhage. The anti OPN monoclonal antibody (0.4 mg/kg) and endoplasmic reticulum stress inhibitor (TUDC) (200 mg/kg) were respectively injected at the same location (unilateral) in the brain parenchyma 1 hour before and 1 hour after hemorrhage.

2.3 Neurological Scoring

Each rat was scored for neurological deficit according to the following scoring criteria: 0: no symptoms of neurological deficit; 1: inability to fully extend the left forelimb; 2: walking in a circle to the left; 3: walking in a tilt to the left; 4: inability to walk spontaneously, spasticity, lethargy, and loss of consciousness. A score of 1 to 3 during postoperative wakefulness indicated successful modeling; scores of 0 and 4 indicated modeling failure and were excluded.

2.4 HE Staining

The cerebral hemorrhage tissue was taken out and washed with running water for several hours 24 hours, 72 hours and 7 days after administration, and dehydrated with 70%, 80% and 90% ethanol solutions at all levels, mixed with pure alcohol and xylene for 15 minutes, and xylene I for 15 minutes and xylene II for 15 minutes (until transparent). Add the mixture of half xylene and half paraffin for 15min, and then add paraffin I and paraffin II for 50-60 minutes respectively. Paraffin embedding and sectioning. Bake paraffin slices, then dewax and hydrate them. Put the slices that have been put into distilled water into hematoxylin aqueous solution for staining for 3min, differentiate them with hydrochloric acid ethanol differentiation solution for 15s, slightly wash them with water, turn blue solution for 15s, wash them with running water, dye them with eosin for 3min, wash them with running water, dehydrate them, make them transparent, seal them, and conduct microscopic examination.

2.5 ELISA Detection

Collect the cerebral hemorrhage and surrounding tissues of rats, take the supernatant from the homogenate, measure the content of iNOS and TNF - α in the cerebral hemorrhage and surrounding tissues of rats by double antibody sandwich method, and refer to the instructions of corresponding ELISA kits for specific steps.

2.6 Statistical Method

SPSS 20.0 software was used for statistical analysis. All experiments were repeated three times, and the quantitative results were expressed as mean ± standard deviation (X ± S). Single factor analysis of variance was used for quantitative value comparison, and S-N-K method was used for pairwise comparison. $P<0.05$ was defined as significant difference.

3 RESULTS

Inflammatory release of RAW264.7 macrophages activated by OPN: In order to explore the effect of OPN on the activation of macrophages induced by high glucose, we used 30 ng/mL OPN to stimulate RAW264.7 macrophages for 24 hours in advance, and then cultured them in 33 mmol/L high glucose medium for 24 hours. Then RT-PCR was used to detect the expression levels of iNOS and IL-1 β in macrophages of each group. The results are shown in Figure 1. Compared with the Control group, In the model group, the expression levels of iNOS and IL-1 β increased significantly. There was a significant difference in the increase of iNOS expression, but there was no significant difference in the increase of IL-1 β expression. Compared with the Model group, the expression levels of iNOS and IL-1 β in the Model+OPN group were further improved, with significant differences.

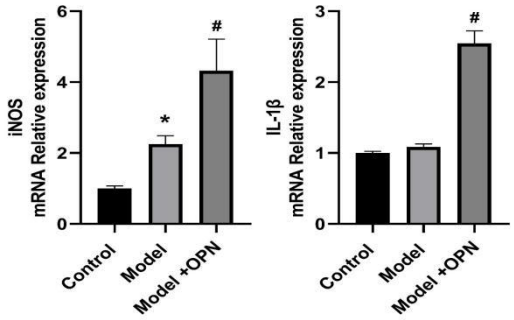

Figure 1 Inflammatory Release of RAW264.7 Macrophages Activated by OPN

Note: RT-PCR was used to detect the expression of iNOS and IL-1 β by RAW264.7 macrophages in each group (* p<0.05 vs. Control, # p<0.05 vs. Model).

3.1 Inflammatory Release of BV2 Cells Activated by OPN

In order to explore the effect of OPN on the activation of microglia induced by high glucose, we used 30 ng/mL OPN to stimulate BV2 cells for 24 hours in advance, and then used 33 mmol/L high glucose medium to culture for 24 hours, and then used RT-PCR to detect the expression levels of TNF - α and IL-1 β in BV2 cells in each group. The results are shown in Figure 2A, compared with the control group, In the model group, the expression levels of TNF - α and IL-1 β increased significantly. Compared with the Model group, the expression levels of TNF - α and IL-1 β in the Model+OPN group were further improved. There was a significant difference in the expression of IL-1 β, but there was no significant difference in the expression of TNF - α. In addition, Western blotting was used to detect the expression level of TNF - α in BV2 cells of each group. The results showed that (Fig. 2B), compared with the Control group, the protein expression level of TNF - α in the Model group increased slightly, with no significant difference. Compared with the Model group, the protein expression level of TNF - α in the Model+OPN group increased slightly, with no significant difference.

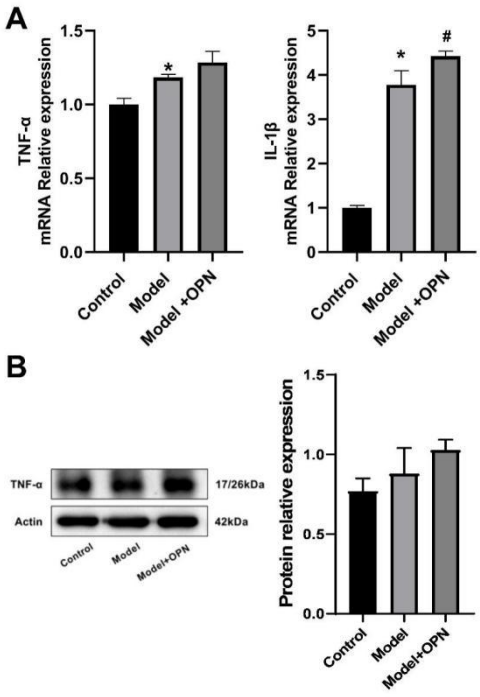

Figure 2 Inflammatory release of BV2 cells activated by OPN

Note: A. The expression levels of TNF - α and IL-1 β in BV2 cells were detected by RT-PCR; B. Western blotting was used to detect the expression level of TNF - α in BV2 cells of each group (* p<0.05 vs. Control, # p<0.05 vs. Model).

OPN inhibitor and endoplasmic reticulum stress inhibitor alleviated neuroinflammation in model rats: we further detected the secretion level of iNOS and TNF - α in intracerebral hemorrhage tissues, as shown in Figure 3. Compared with the control group, the secretion level of iNOS and TNF - α in intracerebral hemorrhage tissues of rats in ICH+HG group was significantly higher, with significant differences, compared with ICH+HG group, In ICH+HG+OPN and ICH+HG+TUDC groups, the secretion levels of iNOS and TNF - α in intracerebral hemorrhage tissues of rats decreased significantly, with significant differences.

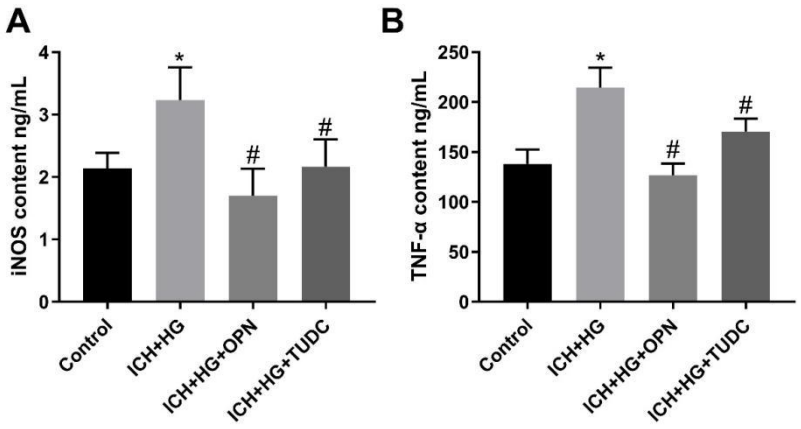

Figure 3 OPN inhibitor and endoplasmic reticulum stress inhibitor alleviate neuroinflammation in model rats
Note: ELISA was used to detect the secretion of iNOS and TNF - α in cerebral hemorrhage tissues of rats in each group (* $p<0.05$ vs. Control, # $p<0.05$ vs. ICH+HG).

4 DISCUSSION

Interleukin-1 beta and iNOS are important factors in the inflammatory activation of macrophages[16, 17]. IL-1 β is a cytokine that can regulate inflammatory response and immune response, and plays an important role in inflammatory activation of macrophages. When macrophages are infected by pathogens or other stimuli, they will release IL-1 β. IL-1 β can activate other immune cells, such as T cells and B cells, and promote the occurrence and development of inflammatory reaction[18]. It can also induce macrophages to produce other inflammatory mediators, such as TNF - α (tumor necrosis factor alpha) and IL-6 (interleukin-6), and enhance the intensity and duration of inflammatory response. In addition, IL-1 β can also affect biological processes such as cell adhesion, migration and proliferation by regulating gene expression[19]. INOS is an enzyme that can catalyze the production of nitric oxide (NO). In the inflammatory activation of macrophages, the expression of iNOS will be induced and increased[20]. Nitric oxide is an important inflammatory mediator that plays an important role in the inflammatory response. Nitric oxide is an important inflammatory mediator that plays an important role in the inflammatory response. It can inhibit the growth and replication of pathogens and enhance the bactericidal ability of macrophages. In addition, nitric oxide can affect biological processes such as cell proliferation, apoptosis and immune response by regulating cell signaling pathways[21]. This study found that high glucose stimulation significantly increased the expression of iNOS and IL-1 β in macrophages, which was close to the research results of Hua et al[22], indicating that high glucose stimulation significantly activated the inflammatory state of macrophages. In addition, the expression levels of iNOS and IL-1 β were further increased after the action of OPN, suggesting that OPN plays an important role in promoting the activation of macrophages induced by high glucose.

Microglia are specialized immune cells in the central nervous system, and they also play an important role in inflammatory activation[23]. When the central nervous system is injured, infected or other pathological stimuli, microglia will activate and release IL-1 β, which can promote the activation and proliferation of microglia. When inflammation occurs, microglia will be stimulated to release a large number of inflammatory mediators, and then continue to activate microglia, forming a positive feedback regulatory cycle[24]. In addition, IL-1 β can also regulate the activity of neurons, affect nerve conduction and neuroinflammatory reaction[25]. TNF - α is a proinflammatory cytokine produced by a variety of cells and plays an important role in regulating the immune and inflammatory response of the body[26]. TNF - α can activate the signal transduction pathway in the cell by binding to the TNF - α receptor on the surface of microglia, leading to the inflammatory response of the cell, including the release of proinflammatory cytokines, increased expression of cell adhesion molecules, and enhanced transcription of inflammation related genes. These reactions further trigger the infiltration and activation of inflammatory cells, forming the inflammatory cycle[27, 28]. This study found that high glucose stimulation significantly increased the expression of TNF - α and IL-1 β in mouse microglia, and the results were consistent with those reported by Zhang et al[29]. It showed that high glucose stimulation significantly activated the inflammatory state of mouse microglia. In addition, the expression levels of TNF - α and IL-1 β further increased after the action of OPN, suggesting that OPN plays an important role in promoting the activation of microglia induced by high glucose.

This study found that OPN inhibitors and ER stress inhibitors can significantly improve the brain tissue pathology of rats with hyperglycemia and intracerebral hemorrhage. With the partial improvement of neural function and the reduction of brain tissue inflammation, it suggests that the effect of OPN inhibitors may be similar to ER stress inhibitors, but this needs further experimental verification[30].

This study preliminarily revealed the potential role of OPN in the pathogenesis of hyperglycemia with cerebral hemorrhage, and found that its role may be related to the activation of inflammatory function of macrophages and microglia. However, this study has some shortcomings. First, in vitro studies found that OPN has a significant role in

promoting the inflammatory activation of macrophages and microglia, However, the results in vitro were not verified by means of immunofluorescence in vivo. In addition, whether the improvement effect of OPN inhibitor on hyperglycemia rats with intracerebral hemorrhage is related to the inhibition of endoplasmic reticulum stress, it is necessary to jointly give OPN inhibitor and endoplasmic reticulum stress agonist to model rats to observe the pathological changes of rats. In future experiments, we will further explore the internal molecular mechanism of OPN regulating inflammatory activation of macrophages and microglia, and better understand its role in the pathogenesis of hyperglycemia combined with cerebral hemorrhage.

To sum up, this study found that OPN may promote hyperglycemia with neuroinflammatory damage after intracerebral hemorrhage by inducing microglia and macrophage inflammation.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

FUNDING

The project was supported by Research Cooperation Project of Guizhou Provincial Department of Science and Technology, [2020]1Y426; Doctoral Research Initiation Fund of Zunyi Medical University Affiliated Hospital, (2019)02.

REFERENCES

[1] Wang Y, Li K, Liu Z, et al. Ethyl Pyruvate Alleviating Inflammatory Response after Diabetic Cerebral Hemorrhage. Curr Neurovasc Res, 2022, 19(2): 196-202.
[2] Diener HC, Hankey GJ. Primary and Secondary Prevention of Ischemic Stroke and Cerebral Hemorrhage: JACC Focus Seminar. J Am Coll Cardiol, 2020, 75(15): 1804-1818.
[3] Chen S, Wan Y, Guo H, et al. Diabetic and stress-induced hyperglycemia in spontaneous intracerebral hemorrhage: A multicenter prospective cohort (CHEERY) study. CNS Neurosci Ther, 2023, 29(4): 979-987.
[4] Sun S, Pan Y, Zhao X, Liu L, Li H, He Y, et al. Prognostic Value of Admission Blood Glucose in Diabetic and Non-diabetic Patients with Intracerebral Hemorrhage. Sci Rep, 2016, 6: 32342.
[5] Chang JJ, Khorchid Y, Kerro A, et al. Sulfonylurea drug pretreatment and functional outcome in diabetic patients with acute intracerebral hemorrhage. J Neurol Sci, 2017, 381: 182-187.
[6] Yang S, Liu Y, Wang S, et al. Association between high serum blood glucose lymphocyte ratio and all-cause mortality in non-traumatic cerebral hemorrhage: a retrospective analysis of the MIMIC-IV database. Front Endocrinol (Lausanne), 2023, 14: 1290176.
[7] Ding H, Jia Y, Lv H, et al. Extracellular vesicles derived from bone marrow mesenchymal stem cells alleviate neuroinflammation after diabetic intracerebral hemorrhage via the miR-183-5p/PDCD4/NLRP3 pathway. J Endocrinol Invest, 2021, 44(12): 2685-2698.
[8] Subhramanyam C S, Wang C, Hu Q, Dheen S T Microglia-mediated neuroinflammation in neurodegenerative diseases. Semin Cell Dev Biol, 2019, 94: 112-120.
[9] Voet S, Prinz M, Van Loo G. Microglia in Central Nervous System Inflammation and Multiple Sclerosis Pathology. Trends Mol Med, 2019, 25(2): 112-123.
[10] De Vlaminck K, Van Hove H, Kancheva D, et al. Differential plasticity and fate of brain-resident and recruited macrophages during the onset and resolution of neuroinflammation. Immunity, 2022, 55(11): 2085-2102 e2089.
[11] Hegdekar N, Sarkar C, Bustos S, et al. Inhibition of autophagy in microglia and macrophages exacerbates innate immune responses and worsens brain injury outcomes. Autophagy 2023, 19(7): 2026-2044.
[12] Icer MA, Gezmen-Karadag M. The multiple functions and mechanisms of osteopontin. Clin Biochem, 2018, 59: 17-24.
[13] Fortis S, Khadaroo RG, Haitsma JJ, et al. Osteopontin is associated with inflammation and mortality in a mouse model of polymicrobial sepsis. Acta Anaesthesiol Scand, 2015, 59(2): 170-175.
[14] Fu H, Liu X, Shi L, et al. Regulatory roles of Osteopontin in lung epithelial inflammation and epithelial-telocyte interaction. Clin Transl Med, 2023, 13(8): e1381.
[15] Manaenko A, Yang P, Nowrangi D, et al. Inhibition of stress fiber formation preserves blood-brain barrier after intracerebral hemorrhage in mice. J Cereb Blood Flow Metab, 2018, 38(1): 87-102.
[16] Kashfi K, Kannikal J, Nath N. Macrophage Reprogramming and Cancer Therapeutics: Role of iNOS-Derived NO. Cells, 2021, 10(11).
[17] Zhu L, Zhao Q, Yang T, et al. Cellular metabolism and macrophage functional polarization. Int Rev Immunol 2015, 34(1): 82-100.
[18] Xia Y, He F, Wu X, et al. GABA transporter sustains IL-1beta production in macrophages. Sci Adv, 2021, 7(15).
[19] Hirano S, Zhou Q, Furuyama A, Kanno S. Differential Regulation of IL-1beta and IL-6 Release in Murine Macrophages. Inflammation, 2017, 40(6): 1933-1943.
[20] McNeill E, Crabtree MJ, Sahgal N, et al. Regulation of iNOS function and cellular redox state by macrophage Gch1 reveals specific requirements for tetrahydrobiopterin in NRF2 activation. Free Radic Biol Med, 2015, 79: 206-216.

[21] Bosca L, Zeini M, Traves PG, Hortelano S. Nitric oxide and cell viability in inflammatory cells: a role for NO in macrophage function and fate. Toxicology, 2005, 208(2): 249-258.
[22] Hua KF, Wang SH., Dong WC., Lin CY, Ho CL, Wu TH. High glucose increases nitric oxide generation in lipopolysaccharide-activated macrophages by enhancing activity of protein kinase C-alpha/delta and NF-kappaB. Inflamm Res, 2012, 61(10): 1107-1116.
[23] Woodburn SC, Bollinger JL, Wohleb ES. The semantics of microglia activation: neuroinflammation, homeostasis, and stress. J Neuroinflammation, 2021, 18(1): 258.
[24] Liu X, Wang Y, Zeng Y, et al. Microglia-neuron interactions promote chronic itch via the NLRP3-IL-1beta-GRPR axis. Allergy, 2023, 78(6): 1570-1584.
[25] Lopez-Rodriguez AB, Hennessy E, Murray CL, et al. Acute systemic inflammation exacerbates neuroinflammation in Alzheimer's disease: IL-1beta drives amplified responses in primed astrocytes and neuronal network dysfunction. Alzheimers Dement, 2021, 17(10): 1735-1755.
[26] Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, et al. The Role of Tumor Necrosis Factor Alpha (TNF-alpha) in Autoimmune Disease and Current TNF-alpha Inhibitors in Therapeutics. Int J Mol Sci, 2021, 22(5).
[27] Bras JP, Bravo J, Freitas J, Barbosa MA, et al. TNF-alpha-induced microglia activation requires miR-342: impact on NF-kB signaling and neurotoxicity. Cell Death Dis, 2020, 11(6): 415.
[28] Wang H, Chen Y, Liu X, et al. TNF-alpha derived from arsenite-induced microglia activation mediated neuronal necroptosis. Ecotoxicol Environ Saf, 2022, 236: 113468.
[29] Zhang J, Li L, Xiu F. Sesamin suppresses high glucose-induced microglial inflammation in the retina in vitro and in vivo. J Neurophysiol, 2022, 127(2): 405-411.
[30] Chen X, Shi C, He M, et al. Endoplasmic reticulum stress: molecular mechanism and therapeutic targets. Signal Transduct Target Ther, 2023, 8(1): 352.