Int J Clin Exp Med 2017;10(6):9065-9072
www.ijcem.com /ISSN:1940-5901/IJCEM0019056

Original Article

Interaction and mechanisms of GRP78 and HSP70 in LPS-treated mice microglia cell

Jian Song[1,2*], Wenjie Lu[1,2*], Li Yang[1], Shun Yao[1], He Huang[1], Yuanzhi He[1], Zheng Liu[1], Yan Yan[1], Youfa Tang[1], Ping Lei[3], Guanxin Shen[3], Lianting Ma[1], Guozheng Xu[1,2]

[1]Department of Neurosurgery, Wuhan General Hospital of PLA, No. 627, Wuluo Road, Hongshan District, Wuhan, Hubei Province, China; [2]The Occurrence and Intervention of Central Nervous System Tumors Key Laboratory of Hubei Province, China; [3]Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. [*]Equal contributors.

Received November 3, 2015; Accepted February 1, 2016; Epub June 15, 2017; Published June 30, 2017

Abstract: Objective: To study the interaction between glucose-regulated protein 78 (GRP78) and heat shock protein 70 (HSP70) in lipopolysaccharide (LPS) treated mouse microglial cells, and explore its mechanism. Methods: mouse microglia (BV2) was the research object, bacterial lipopolysaccharide (LPS) infected BV2 cells were used to observe the active status which were stimulated by LPS. We analyzed TLR2, TLR4 expression level of the infected cells. We also investigated LPS stimulated BV2 cell. Expression level of IL-1β, TNF-α, IFN-β and other cytokines were observed. When GRP78 and HSP70 were injected into BV2 LPS-stimulated cells, changes of nitric oxide synthase (iNOS) and reactive oxygen species (ROS) were observed. Results: Immunofluorescence staining showed that surface of normal BV2 cell appeared red fluorescent particles, with some activation state. After LPS, GRP, HSP70 protein and LPS treatment, microglia activation state had different degrees of incensement. Expression results of TLR2 and TLR4 showed that after LPS stimulated TLR2 expression showed significant difference ($P>0.05$) compared with the normal cells, while expression of GRP78 and HSP70 injected LPS stimulated TLR2 showed significant difference compared with the normal group and the LPS-treated group with significant difference ($P<0.05$); expression of TLR4 in LPS stimulated group, GRP78 + LPS group and HSP70 + LPS group showed statistical difference ($P<0.05$) compared with the normal cells. ELISA results showed that the IL-1β had significant difference between five experimental groups ($P<0.05$), while it was increased significantly in HSP70 + LPS treatment group, and LPS treatment group was followed; TNF-α in normal cells group showed statistical significance ($P<0.05$) compared with the rest four experimental groups; IFN-β in normal cells and LPS-treated group showed statistical significance compared with GRP78 + LPS group and HSP70 + LPS group ($P<0.05$), but there was no difference when compared with LPS stimulated neurons. Expression of iNOS, compared with the five experimental groups, had significant difference ($P<0.05$), while HSP70 + LPS treatment group showed the most obvious incensement. The results of ROS had significant difference ($P<0.05$) compared with the results of five experimental groups, while HSP70 + LPS treatment group also showed the most obvious incensement. Conclusion: LPS stimulation can cause microglial activation in mice. Through TLR4 pathway, IL-1β, TNF-α and other cytokines can be secreted extensively, leading iNOS and reactive oxygen species (ROS) increased. Meanwhile, GRP78 protein may play a more important role in the inhibition of LPS-stimulated microglia inflammation and other stress.

Keywords: GRP78, HSP70, LPS, microglia

Introduction

Microglia is one of the main organism immune effector cells, widely distributed in the central nervous system, accounting for about 20% of the total number of glial cells [1]. Although research shows that early activated microglia has a protective and repair effect on neurons, excessive activation of microglia is able to produce inflammatory substances, causing neuronal damage and inducing the occurrence and development of central nervous system diseases [2]. Microglia activation and its related inflammation, immune response play an important role in the development of many neurological diseases, in which the inflammatory receptor signaling pathway plays an important role [3, 4].

Heat shock proteins (HSP), also known as stress proteins, are a kind of evolutionarily highly-conserved proteins, highly expressed

when HSP genes are activated by some stressors, such as the environment, high temperature, ischemia, infection, trauma, drugs and other conditions [5], playing an important role in preventing the body from being damaged by stress factors; HSP can improve the stress ability of cells to resist the influence of various damaging factors, playing a role in protecting cells [6]. Glucose-regulated protein 78 (GRP78), also known as immunoglobulin heavy chain binding protein, is a member of the HSP70 family. As a stress protein, GRP78 is highly expressed under stress to maintain a stable environment within cells, which can extend cell survival in the stimulation of a variety of harmful factors [7-9].

Gram-negative bacteria lipopolysaccharide (LPS) is an important structural component of the bacterial cell wall, which is also an important aspect of the pathogenic mechanisms; it is an important microglia activator, commonly used in the construction and research of experimental models [10]. Previous report showed that LPS stimulated signal transduction path for microglia cells had been inveatigated [11], but the influence and role of LPS pathogenesis of heat shock proteins and signal transduction pathways had not been studied. This was the starting point of article, and we explored the role and significance of heat shock protein GRP78 and HSP70 family members in LPS stimulated mice microglia cells.

Materials and methods

After GRP78 and HSP70 were injected, activation status of LPS-stimulated BV2 cells

After GRP78 and HSP70 were injected, activation status of LPS-stimulated BV2 cells was detected by immunofluorescence stainingThe cells were divided into 4 groups: normal group; LPS-treated group; GRP78 + LPS group; HSP70 + LPS group; 3 samples per group. In the plates, the prepared cell climbing film was immersed and washed with PBS, then fixed with 4% paraformaldehyde; after washing, 6% normal goat serum was dropped in cell climbing film to close it at room temperature for 60 min. Primary antibody incubation: a sufficient amount of diluted antibody (primary antibody: rabbit CD11b, namely OX-42; antibody concentration: 1:500) was dropped on each film, and then the film was put into the wet box and incubated at 4°C overnight; secondary antibody incubation: diluted fluorescent secondary antibody was dropped (secondary antibody: goat anti-rabbit Alexa Fluor 647; second antibody concentration: 1:1000), ansd then cell climbing film was incubated in wet box at 37 °C for 60 min; Nucleus counterstaining: after dark DAPI incubation for 5 min, nuclear staining of specimens was performed. Then films were mounted and image acquisition was performed with a laser scanning confocal microscope.

After GRP78 and HSP70 were injected into LPS-stimulated BV2 cells, the expression of TLR2 and TLR4

QRT-PCR was used to detect the expression of TLR2 and TLR4 after GRP78 and HSP70 were injected into LPS-stimulated BV2 cells. The cells were divided into 4 groups: 1-control BV2 group; 2LPS-treated BV2 group; 3 -GRP78 + LPS treatment group; 4-HSP70 + LPS treatment group. Based on the amplified gene, specific primers were designed (**Table 1**), and were detected using SYBR Green I fluorescent dye method.

Total RNA was extracted using TRIzol method; the RNA precipitate was dissolved in an appropriate amount (20-50 ul) of RNase-free water, stored at -70°C. Reverse transcription system was prepared as follows: 2 × RT buffer 10 ul, 6N random primer (100 pmol/ul) 1 ul, RT-mix 1 ul, template (RNA) 5 ul, DEPC water 3 ul; reaction parameters were: 25°C 10 min, 42°C 50 min, 85°C 5 min. Fluorescence quantitative detection system was: 2 × PCR buffer 25 ul, Primers (25 pmol/ul) 1 ul × 2, SYBR green I 0.5 ul, template (cDNA) 2 ul, DEPC water 20.5 ul; reaction parameters were: 94°C 4 min; 94°C 20 s, 60°C 30 s, 72°C 30 s, 35 cycles; signal was detected at 72°C.

After GRP78 and HSP70 were injected into LPS-stimulated BV2 cells, the expression of IL-1β, TNF-α and IFN-β

Double antibody sandwich ELISA assay was used to detect IL-1β, TNF-α and IFN-β. Standard curve of culture supernatant was prepared. The cells were divided into five groups: 1-blank group; 2-control group (LPS-treated group); 3-GRP78 + LPS treatment group; 4-HSP70 + LPS treatment group; 5-direct LPS stimulation group. Prepare the desired reagents and working standards. 50 ul of standard and sample was added, ensuring continuous sampling, without break. Loading process was completed within 15 minutes (standard con-

Table 1. Primers and sequences in QRT-PCR detection

		Primers	Sequences	Length
Tlr2	Upstream	mTlr2F	TTCACCACTGCCCGTAGATG	148 bp
ID: 24088	Downstream	mTlr2R	GGTACAGTCGTCGAACTCTACCTC	
Tlr4	Upstream	mTlr4F	CAGCAGGTGGAATTGTATCGC	173 bp
ID: 21898	Downstream	mTlr4R	CAGGTCCAAGTTGCCGTTTC	
Internal reference	Upstream	m actin f	GAGACCTTCAACACCCCAGC	263 bp
	Downstream	m actin r	ATGTCACGCACGATTTCCC	

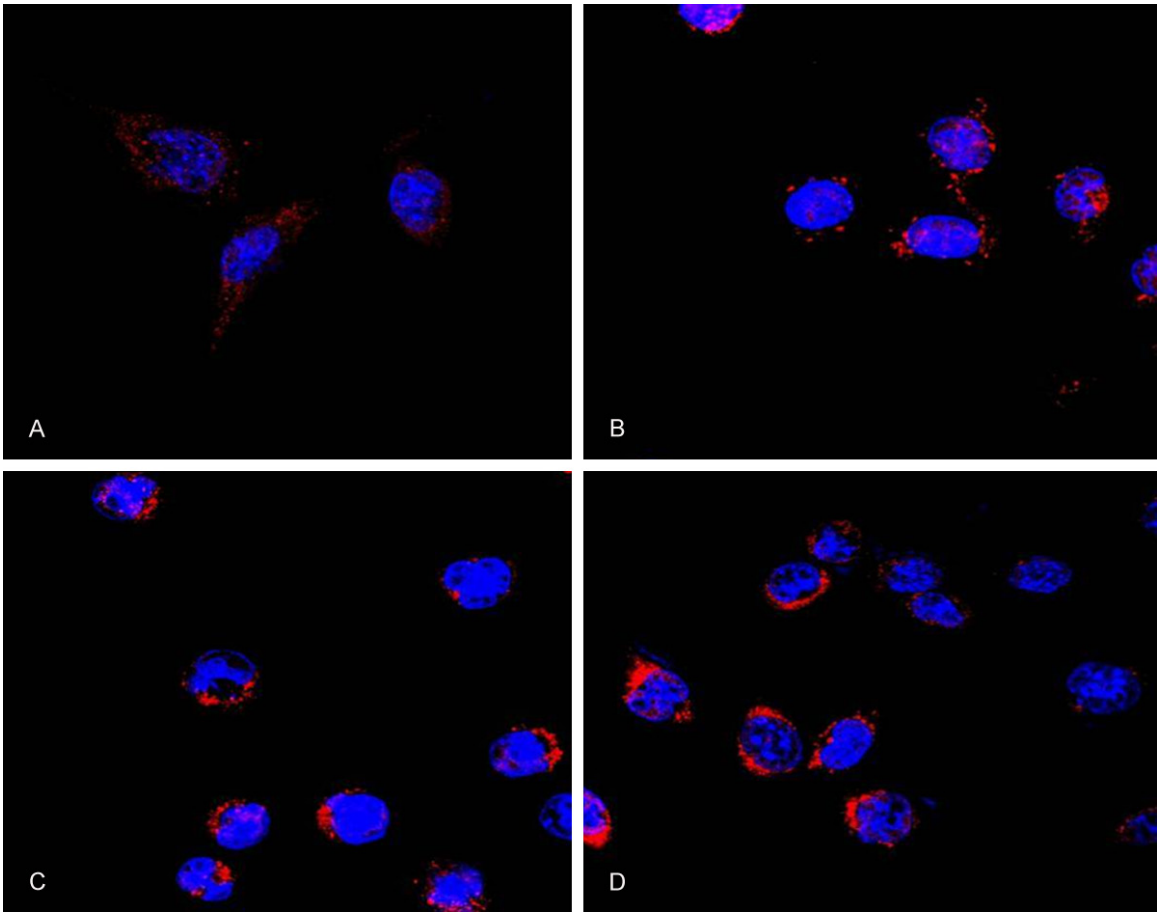

Figure 1. The red phosphor particles were the activated microglia, while blue phosphor particles were the nuclei. A. Control group; B. LPS group; C. GRP78 + LPS group; D. HSP70 + LPS group.

centrations were 500 pg/ml, 200 pg/ml, 80 pg/ml, 32 pg/ml, 12.8 pg/ml, 5.12 pg/ml, 2.05 pg/ml). 50 ul detection antibody was added into each well. Closure plate membrane was used to seal plate, and cells were incubated at room temperature for 2 hours. After washing, 100 ul horseradish peroxidase-labeled streptavidin-biotin was added into each well. After re-washing, 100 ul chromogenic substrate TMB was added to each well, and cells were incubated darkly at room temperature for 10-30 minutes. Then 100 ul of stop solution was added to each well; within 30 minutes, OD values at 450 nm were measured by a microplate reader.

After GRP78 and HSP70 were injected into LPS-stimulated BV2 cells, the expression of iNOS

Western blot was used to detect the expression of iNOS after GRP78 and HSP70 were injected into LPS-stimulated BV2 cells. The cells were divided into five groups: 1-blank group; 2-control

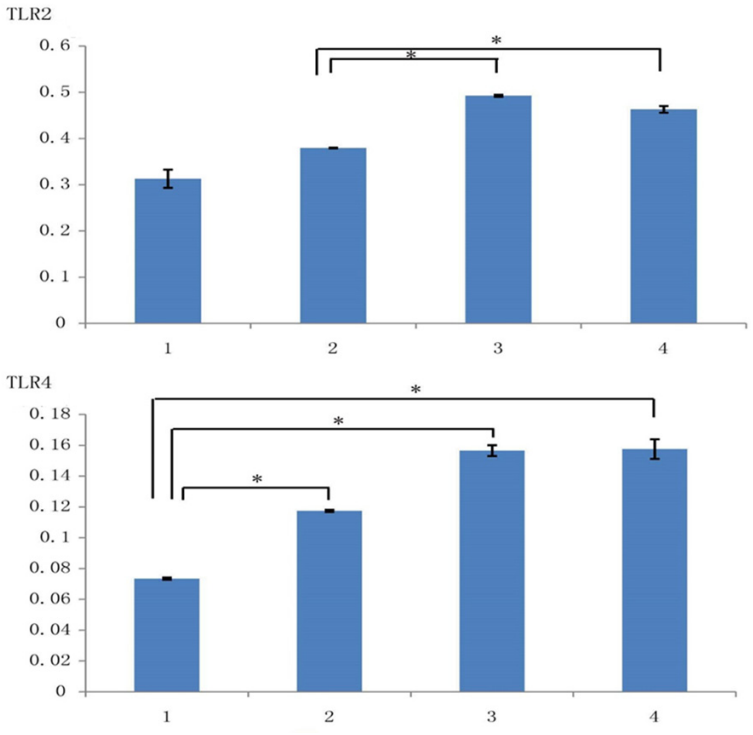

Figure 2. TLR2 and TLR4 expression in GRP78 and HSP70 protein separately injected LPS stimulated BV2 cells. 1: control group BV2; 2: LPS-treated group; 3: GRP78 + LPS-treated group; 4: HSP70 + LPS treated group. Note: *represents the difference between the two groups with statistical significance ($P<0.05$).

group (LPS-treated group); 3-GRP78 + LPS treatment group; 4-HSP70 + LPS treatment group; 5-direct LPS stimulation group. Protein concentrations were determined using the BCA method. Then samples were subjected to SDS-PAGE and WB detection: according to procedures of vertical electrophoresis, glue tank was assembled; 10% separating gel and 4% stacking gel were formulated. The sample was mixed with 5 × SDS sample buffer at a ratio of 4:1; after mixing, the mixture was put in boiling water for 5 min to denature the proteins. After enough electrophoretic liquid was added, sample containing the same amount of protein was loaded and subjected to electrophoresis. Film transfer apparatus was assembled, meanwhile the electrophoretic liquid was replaced with the transfer liquid. The current was adjusted to a constant current of 200 mA; transfer was performed for about 1 hour. After film transfer, fim was washed in TBST for 1 minute, and then blocked with blocking solution. The corresponding antibody was diluted in blocking buffer to a certain concentration (1:500); the internal reference antibody (GAPDH) was diluted to a final concentration of 1:3000; then sample was incubated with it warmly for 1.5 hours or incubated at 4°C overnight. Secondary antibody was diluted by blocking solution to a certain concentration (1:3000), and then sample was incubated with it for 1.5 hours. Finally, chemiluminescent, developing and fixing were performed. After completion of the experiment, film scanning was performed, and the gray value of target band was analyzed by UVP gel image processing system Labworks4.6 software.

After GRP78 and HSP70 were injected into LPS-stimulated BV2 cells, the changes of reactive oxygen species (ROS) were detected

Flow cytometry was used to detect the changes of reactive oxygen species (ROS) after protein GRP78 and HSP70 were injected into LPS-stimulated BV2 cells. The cells were divided into five groups: 1-blank group; 2-control group (LPS-treated group); 3-GRP78 + LPS treatment group; 4-HSP70 + LPS treatment group; 5-direct LPS stimulation group. Cells in logarithmic growth phase were collected and cell concentration was adjusted to 1×10^5/mL; then cells were seeded in 6-well plates and cultured until adherent. Cells were treated according to experimental groups before loading fluorescent probe: DCFH-DA was added in the medium; working concentration of DCFH-DA was 60nM, and then the cells were incubated at 37°C for 40 min. Cells were collected: Cells were trypsinized (0.25% trypsin, 2-3 min); the culture medium was added to terminate the digestion; cell suspension was prepared; after 5-10 minutes of 1000 g centrifugation, the cells were collected, washed with PBS, re-centrifuged and collected; then the cells were re-suspended in PBS and detected by flow cytometry. Wavelength setting: optimum excitation wavelength 500,485 (500 ± 15 nm), the optimum emission wavelength 525 (530 ± 20 nm). It can also be detected in

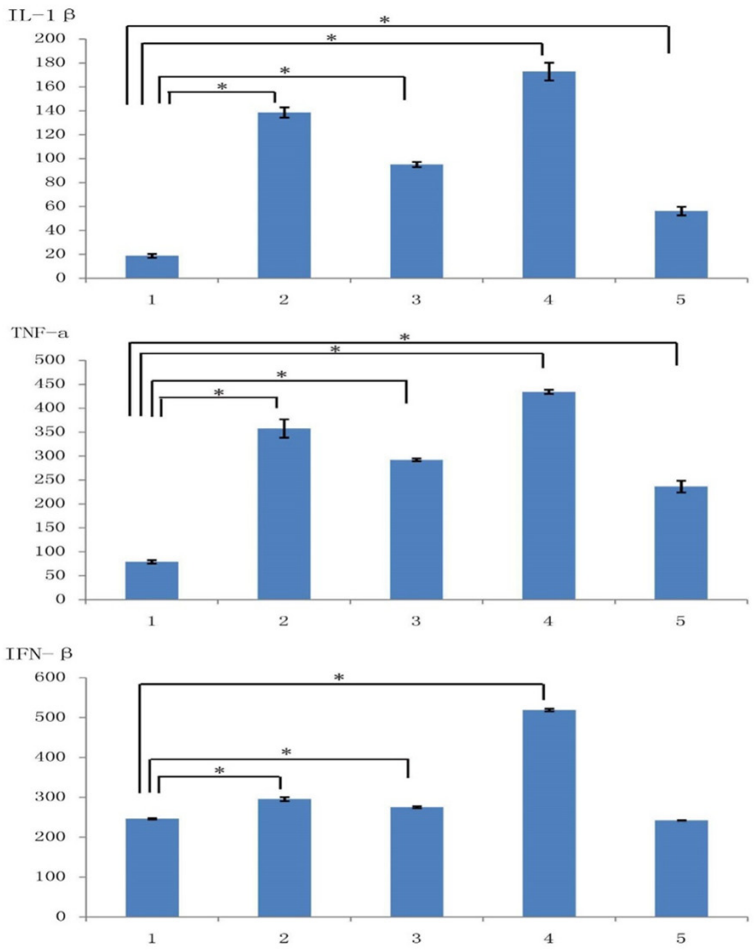

Figure 3. GRP78 and HSP70 protein injected LPS stimulated BV2 cells, and changes of cytokine. 1: control group BV2; 2: LPS-treated group; 3: GRP78 + LPS-treated group; 4: HSP70 + LPS treated group; 5: LPS directly stimulated neurons group. Note: *represents the difference between the two groups with statistical significance (P<0.05). The figure marked the differences between the control group and the rest of the group only. Other differences between the groups were describted in the text.

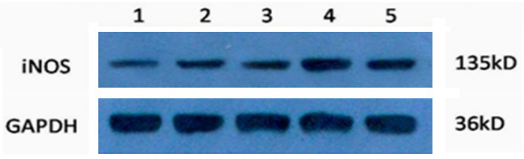

Figure 4. iNOS and internal reference grayscale images.

accordance with FITC fluorescence detection conditions.

Statistical analysis

SPSS17.0 was used for statistical analysis; quantitative variables were analyzed using analysis of variance and t test. P<0.05 was considered statistically significant.

Results

Activated state of GRP78 and HSP70 injected LPS stimulated BV2 cells separately

Immunofluorescence staining showed that surface of normal BV2 cell appear red fluorescent particles, with some activation state. After LPS, GRP, HSP70 protein and LPS treatment, microglia activation state had different degrees of incensement, with the enhancing red fluorescent particles, as shown in **Figure 1A-D**.

TLR2 and TLR4 expression in GRP78 and HSP70 protein injected LPS stimulated BV2 cells

Expression results of TLR2 and TLR4 showed that after LPS stimulated TLR2 expression showed significant difference (P>0.05) compared with the normal cells, while expression of GRP78 and HSP70 injected LPS stimulated TLR2 showed significant difference compared with the normal group and the LPS-treated group with significant difference (P<0.05); expression of TLR4 in LPS stimulated group, GRP78 + LPS group and HSP70 + LPS group showed statistical difference (P<0.05) compared with the normal cells, as shown in **Figure 2**.

Changes of cytokines

ELISA results showed that the IL-1β had significant difference between five experimental groups (P<0.05), while it was increased significantly in HSP70 + LPS treatment group, and LPS treatment group was followed; TNF-α in normal cells group showed statistical significance (P<0.05) compared with the rest four

Table 2. iNOS and internal reference value changes for each gradation

	Lanes	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Grayscale value	iNOS	110.78	175.23	178.82	318.92	250.8
	GAPDH	521.17	519.49	519.86	503.76	493.11
	iNOS/GAPDH	0.21256	0.33731	0.343977	0.633079	0.508609

Note: 1: BV2 control group; 2: LPS-treated group; 3: GRP78 + LPS-treated group; 4: HSP70 + LPS treated group; 5: LPS directly stimulated neurons group.

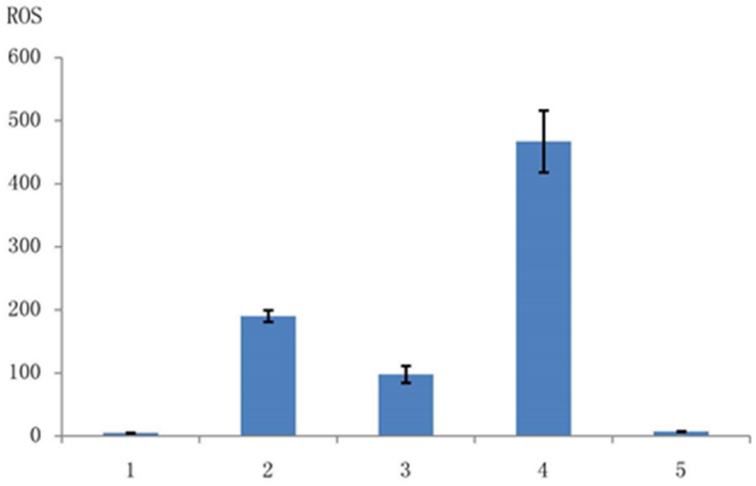

Figure 5. Changes of reactive oxygen species (ROS) between experimental groups. 1: BV2 control group; 2: LPS-treated group; 3: GRP78 + LPS-treated group; 4: HSP70 + LPS treated group; 5: LPS directly stimulated neurons group.

experimental groups; IFN-β in normal cells and LPS-treated group showed statistical significance compared with GRP78 + LPS group and HSP70 + LPS group ($P<0.05$), but there was no difference when compared with LPS stimulated neurons, as shown in **Figure 3**.

GRP78 and HSP70 protein injected LPS stimulated BV2 cells, and expression of iNOS

Expression of iNOS in five experimental groups had significant difference ($t = 5.538$, $P<0.05$), while the HSP70 + LPS treatment group increased the most significantly, as shown in **Figure 4** and **Table 2**.

GRP78 and HSP70 protein injected LPS stimulated BV2 cells, and changes of ROS

Samilarily, ROS in the five experimental groups showed significant difference ($P<0.05$). Between any two experimental groups there were statistical difference. HSP70 + LPS treatment group increased most significantly, and LPS-treated group was followed, as shown in **Figure 5**.

Discussion

The current study concluded that, LPS activating microglia is an extremely complex process of signal transduction; in the signaling pathway activated by LPS, Toll-like receptor (TLR) is the main participant [12]. Toll-like receptor is an evolutionarily highly-conserved type I transmembrane protein, consisting of three components: an extracellular N-terminal, capable of recognizing extracellular pathogens and tissue damage signal; transmembrane domain; intracellular C-terminal, TIR binding area [13]. TLR as a common pattern recognition receptor, can combine with pathogen-associated molecular patterns (such as LPS), and start the non-specific immune effector mechanisms of cells. Microglia is the most important immune cell in the central nervous system; its most important role is to be involved in the immune response of central nervous system as antigen-presenting cells [14]. Microglia TLR can recognize pathogen-associated molecular patterns, and constitutively express MHC II; after cell activation, it can induce high expression of MHC II, ICAM-1 and other immune costimulatory molecules and adhesion molecules, and activate T cells [15]. Therefore, microglia plays a very important role in anti-inflammation and anti-infection in the nervous system. The results of this study show that, after acting on mouse microglial cells, LPS can mediate innate immune response mechanism to promote the differentiation and maturation of microglial cells. At the same time, our findings indicate that, LPS affects microglia primarily through TLR4 pathway; in the

experimental results, there is no difference in TLR2 pathway between normal group and LPS stimulation group, while there are differences in TLR4 pathway between normal group and other experimental groups, indicating that after LPS stimulation, BV2 works primarily through TLR4. This result is similar to the previous result. Hines et al [16] used TLR4 receptor to prevent the LPS-induced microglial cell activation and inflammatory cytokine release, stating the importance of TLR4 signaling pathway; Yao et al [17] suggested that TLR4 receptor mediated the hypoxia-induced microglia activation and inflammatory cytokine release. TLR4 accepted the action of bacterial endotoxin and heat shock proteins and other damage factors to trigger the generation and expression of various inflammatory cytokines by activating downstream NF-κB activity, leading to inflammation.

In this study, the activated microglia not only can produce inflammatory cytokines (IL-1β, TNF-α and IFN-β), but also can generate free radicals such as ROS and iNOS; these free radicals are capable of causing poisoning state of nervous system [18]. In mammalian cells, after activated by TLR signals, NF-κB can induce the synthesis of ROS and RNS [11]. TLR4 was activated by various ligands, resulting in the expression of iNOS gene and NO generation by microglia [19].

At the same time, we also found that that in BV2 cell response against stress, GRP78 protein played a more important role in suppressing inflammation and other processes [20-22]. The present experimental results showed that in the experimental group with GRP78 protein injection, the secretion levels of cytokines IL-1β, TNF-α and IFN-β were lower than those in LPS group and HSP70 protein injection group; this effect can reduce the body's inflammatory reaction, to avoid the damage to the nerve cells by cytokine tide. On the other hand, GRP78 protein can significantly reduce the amount of iNOS and ROS, slowing the occurrence and development of inflammation.

Disclosure of conflict of interest

None.

Address correspondence to: Guozheng Xu and Lianting Ma, Department of Neurosurgery, Wuhan General Hospital of PLA, No. 627, Wuluo Road, Hongshan District, Wuhan, Hubei Province, China. Tel: +86-027-87740350; Fax: +86-027-87740350; E-mail: xuguozhe091@126.com (GZX); Tel: +86-027-50772493; Fax: +86-027-50772493; E-mail: mlt1937@163.com (LTM)

References

[1] Farber K, Kettenmann H. Physiology of microglial cells. Brain Res Brain Res Rev 2005; 48: 133-143.

[2] Floden AM, Combs CK. Microglia repetitively isolated from in vitro mixed glial cultures retain their initial phenotype. J Neurosci Methods 2007; 164: 218-224.

[3] Barker AJ, Ullian EM. New roles for astrocytes in developing synaptic circuits. Commun Integr Biol 2008; 1: 207-211.

[4] Bessis A, Bechade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. Glia 2007; 55: 233-238.

[5] Kim KS, Lee AS. The effect of extracellular Ca2+ and temperature on the induction of the heat-shock and glucose-regulated proteins in hamster fibroblasts. Biochem Biophys Res Commun 1986; 140: 881-887.

[6] Leu JI, Pimkina J, Frank A, Murphy ME, George DL. A small molecule inhibitor of inducible heat shock protein 70. Mol Cell 2009; 36: 15-27.

[7] Langer R, Feith M, Siewert JR, Wester HJ, Hoefler H. Expression and clinical significance of glucose regulated proteins GRP78 (BiP) and GRP94 (GP96) in human adenocarcinomas of the esophagus. BMC Cancer 2008; 8: 70.

[8] Kim SK, Kim YK, Lee AS. Expression of the glucose-regulated proteins (GRP94 and GRP78) in differentiated and undifferentiated mouse embryonic cells and the use of the GRP78 promoter as an expression system in embryonic cells. Differentiation 1990; 42: 153-159.

[9] Dorner AJ, Wasley LC, Kaufman RJ. Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. EMBO J 1992; 11: 1563-1571.

[10] Roth J, Blatteis CM. Mechanisms of fever production and lysis: lessons from experimental LPS fever. Compr Physiol 2014; 4: 1563-1604.

[11] Shishido T, Nozaki N, Takahashi H, Arimoto T, Niizeki T, Koyama Y, Abe J, Takeishi Y, Kubota I. Central role of endogenous Toll-like receptor-2 activation in regulating inflammation, reactive oxygen species production, and subsequent neointimal formation after vascular injury. Biochem Biophys Res Commun 2006; 345: 1446-1453.

[12] Lehnardt S. Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. Glia 2010; 58: 253-263.
[13] Sierra A, Gottfried-Blackmore AC, McEwen BS, Bulloch K. Microglia derived from aging mice exhibit an altered inflammatory profile. Glia 2007; 55: 412-424.
[14] Trapp BD, Wujek JR, Criste GA, Jalabi W, Yin X, Kidd GJ, Stohlman S, Ransohoff R. Evidence for synaptic stripping by cortical microglia. Glia 2007; 55: 360-368.
[15] Acarin L, Gonzalez B, Castellano B. Neuronal, astroglial and microglial cytokine expression after an excitotoxic lesion in the immature rat brain. Eur J Neurosci 2000; 12: 3505-3520.
[16] Hines DJ, Choi HB, Hines RM, Phillips AG, MacVicar BA. Prevention of LPS-induced microglia activation, cytokine production and sickness behavior with TLR4 receptor interfering peptides. PLoS One 2013; 8: e60388.
[17] Yao L, Kan EM, Lu J, Hao A, Dheen ST, Kaur C, Ling EA. Toll-like receptor 4 mediates microglial activation and production of inflammatory mediators in neonatal rat brain following hypoxia: role of TLR4 in hypoxic microglia. J Neuroinflammation 2013; 10: 23.
[18] Li L, Zhang J, Jin B, Block ER, Patel JM. Nitric oxide upregulation of caspase-8 mRNA expression in lung endothelial cells: role of JAK2/STAT-1 signaling. Mol Cell Biochem 2007; 305: 71-77.
[19] Goos M, Lange P, Hanisch UK, Prinz M, Scheffel J, Bergmann R, Ebert S, Nau R. Fibronectin is elevated in the cerebrospinal fluid of patients suffering from bacterial meningitis and enhances inflammation caused by bacterial products in primary mouse microglial cell cultures. J Neurochem 2007; 102: 2049-2060.
[20] Little E, Ramakrishnan M, Roy B, Gazit G, Lee AS. The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. Crit Rev Eukaryot Gene Expr 1994; 4: 1-18.
[21] Wang M, Wang P, Peng JL, Wu S, Zhao XP, Li L, Shen GX. The altered expression of glucose-regulated proteins 78 in different phase of streptozotocin-affected pancreatic beta-cells. Cell Stress Chaperones 2009; 14: 43-48.
[22] Allagnat F, Fukaya M, Nogueira TC, Delaroche D, Welsh N, Marselli L, Marchetti P, Haefliger JA, Eizirik DL, Cardozo AK. C/EBP homologous protein contributes to cytokine-induced pro-inflammatory responses and apoptosis in beta-cells. Cell Death Differ 2012; 19: 1836-1846.