European Review for Medical and Pharmacological Sciences 2018; 22: 6042-6048

Effects of IL-1β on hippocampus cell apoptosis and learning ability of vascular dementia rats

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Abstract. – OBJECTIVE: Vascular dementia (VD) is a type of memory, cognition, and behavior disorder caused by ischemic stroke or hemorrhagic stroke. It is a common pathogenesis of dementia that is only second to Alzheimer's disease. Inflammation plays a key role in VD. Interleukin-1β (IL-1β) is a kind of pro-inflammatory cytokine, while its mechanism in VD occurrence and development is still unclear.

MATERIALS AND METHODS: The healthy male rats were randomly divided into three groups, including sham group, VD model group (established by bilateral common carotid artery ligation), and IL-1β group (treated by IL-1β monoclonal antibody intracerebroventricular injection on based on model group). Rat learning ability was evaluated by Morris water maze assay. IL-1β expression in brain tissue and peripheral blood was examined by using Real Time-PCR and enzyme-linked immunosorbent assay (ELISA), respectively. Hippocampus apoptosis was detected by caspase 3 activity detection kit. B-cell lymphoma-2 (Bcl-2) and p38 mitogen-activated protein kinase (MAPK) protein levels were assessed by Western blot assay.

RESULTS: IL-1β expression was increased, caspase 3 activity was enhanced, Bcl-2 level was declined, and p-P38 phosphorylation was elevated in brain tissue and peripheral blood from VD model group compared to sham group ($p<0.05$). IL-1β monoclonal antibody significantly reduced IL-1β expression, improved learning ability, attenuated caspase 3 activity, increased Bcl-2 level, and declined p-P38 expression in VD rats compared to model group ($p<0.05$).

CONCLUSIONS: IL-1β can delay VD occurrence and development through the P38-MAPK signaling pathway to regulate cell apoptosis and improve learning ability.

Key Words:
Vascular dementia, IL-1β, P38MAPK, Apoptosis, Caspase 3, Bcl-2.

Introduction

Cerebrovascular accident and disease, including ischemic stroke and hemorrhage stroke, can induce vascular dementia (VD). It is mainly caused by ischemia and hypoxia, leading to progressive and acquired cognitive and behavior disorder, which seriously affects linguistic function, memory, and space skill[1,2]. As a common cause of dementia, VD exhibits a high morbidity only second to Alzheimer disease. It is widely concerned in clinic because of its serious influence on quality of life, economic and mental pressure, and society burden[3,4]. Following the increase of the older population, VD prevention is an important problem needs to be solved in geriatrics and related disciplines[5,6]. High incidence rate of atherosclerosis, hypertension, and cardiovascular and cerebrovascular disease in our country leads to high VD morbidity around the world[7,8]. Multiple factors may induce VD, while the specific mechanism still needs further elucidation. As a common type of senile dementia, VD is the most promising type of the prevention[9,10]. It was showed that inflammation and oxidative stress are closely associated with VD[11,12]. There is still a lack of effective treatment target for VD. Therefore, investigation of the pathogenesis of VD is of great significance to find a new molecular target for the treatment.

Inflammation is considered to be an important mechanism to induce VD occurrence and development, which attracts much attention[13]. IL-1β is a key inflammatory cytokine that can induce leukocytes and inflammatory cells adhesion and accumulation in microvessels[14,15]. IL-1β can induce arterial reocclusion, neuron injury, and cell apoptosis to aggravate VD[16]. Therefore, this study explored the role and related mechanism of IL-1β on VD.

Materials and Methods

Experimental Animals

Healthy male Wistar rats at two months old and weighted 250 ± 20 g were purchased from Harbin

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Medical University Experimental Animal Center (Harbin, China) and raised in specific pathogen free (SPF) grade experimental animal center. The raising condition contained temperature at 21°C ± 1°C, relative humidity at 50%-70%, and 12 h day/night cycle.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of The Fifth Affiliated Hospital of Harbin Medical University.

Main Materials and Instruments

10% chloral hydrate was purchased from Zhpharma (Shanghai, China). IL-1β monoclonal antibody was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). IL-1β enzyme-linked immunosorbent assay (ELISA) kit was purchased from R&D (Minneapolis, MN, USA). Polyvinylidene difluoride (PVDF) membrane was derived from Pall Life Sciences (Covina, CA, USA). Caspase 3 activity detection kit and Western blot related reagents were provided by Beyotime Biotechnology (Shanghai, China). Enhanced chemiluminescence (ECL) reagent was purchased from Amersham Biosciences (Piscataway, NJ, USA). Rabbit anti-mouse p-P38 MAPK monoclonal antibody, Bcl-2 monoclonal antibody, and goat anti-rabbit horseradish peroxidase (HRP) labeled IgG secondary antibody were provided by Cell Signaling Technology (Beverly, MA, USA). RNA extraction kit and reverse transcription kit were purchased from ABI (Foster City, CA, USA). ABI 7500 Real Time-PCR amplifier was derived from ABI (Foster City, CA, USA). Microscopic surgery instrument was purchased from Suzhou medical apparatus factory (Suzhou, China). Multi-Parameter Monitor animal physiological monitor, electroencephalograph (EEG) recorder, and YC-2 stimulator were purchased from Yuyanbio (Shanghai, China). DNA amplifier was obtained from PE Gene Applied Biosystems (Mode: Gene Amp PCR System 2400, Foster, CA, USA). Labsystem Version1.3.1 micro-plate reader was provided by Bio-Rad Laboratories (Hercules, CA, USA). Other reagents were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China).

Experimental Animal Grouping and Treatment

The healthy male rats were randomly divided into three groups, including sham group, VD model group established by bilateral common carotid artery ligation, and IL-1β group treated by IL-1β monoclonal antibody intra-cerebroventricular injection on based on model group.

Rat VD Model Establishment

Rat VD model was established by bilateral common carotid artery ligation[17]. The rat was anesthetized by 0.35 ml/100 g 10% chloral hydrate abdominal injection and fixed on stereotaxic apparatus. Then, the neck skin was disinfected and the incision was made on the neck midcourt line. The muscle and connective tissues were separated to isolate the bilateral common carotid artery for ligation. The vagus nerve was protected to avoid damage. Rat breathes and heart rates were observed. 10^4 U gentamycin was used for three days after surgery to prevent infection. The rats in the sham group received the same treatment without bilateral common carotid artery ligation.

Morris Water Maze Test

Navigation test and space probe test in the Morris water maze test were applied[18]. The time from entry water to reach underwater platform was recorded as escape latency. Rat swimming time and the times of crossing platform to search platform within 120 s were recorded to test rat learning and memory abilities.

Sample Collection

A total of 2 ml blood was extracted from the rat caudal vein and centrifuged at 2000 r/min for 5 min. The supernatant was stored at -20°C. The hippocampus tissue was extracted and stored at -80°C.

Real Time-PCR

Total RNA was extracted from hippocampus tissue by TRIzol and reverse transcribed to complementary DNA (cDNA). The primers were designed using PrimerPremier 6.0 software (Table I) and synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). Real-time PCR was performed at 56°C for 1 min, followed by 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as internal reference. The relative expression of mRNA was calculated by $2^{-\Delta Ct}$ method.

ELISA

ELISA was used to test IL-1β content in the serum. A total of 50 μl diluted standard substance were added to each well to establish a standard curve. Next, the plate was added with 50 μl sample and washed for five times. Then, the plate was incubated in 50 μl conjugate reagent at 37°C for 30 min. After washed five times, the plate was treated with 50 μl color agent A and B at 37°C

Table I. Primer sequences.

Gene	Forward 5′-3′	Reverse 5′-3′
GAPDH	AGAGTACCTTGCTTCTGGG	TAATGATAGGTGACCCCTGGT
IL-1β	CCCTGCCCTGTATTACAATC	GATGGTATTTATGATATCCC

avoid of light for 30 min. At last, the plate was added with 50 μl stop buffer to stop the reaction and tested at 450 nm to obtain the optical density (OD) value. The OD value of the standard substance was used to prepare the linear regression equation, which was adopted to calculate the concentration of samples.

Caspase 3 Activity Detection

Caspase 3 activity was tested according to the manual. The cells were digested by trypsin and centrifuged 5 minutes at 600 ×g and 4°C. Next, the cells were added with 2 mM Ac-DEVD-pNA and detected at 405 nm to calculate caspase 3 activity.

Western Blot

The hippocampus tissues were added with radioimmunoprecipitation assay (RIPA) containing protease inhibitor and cracked on ice for 15-30 min. Next, the tissues were treated by ultrasound at 5 s for 4 times and centrifuged 15 minutes at 10000 ×g. The protein was transferred to a new Eppendorf (Ep) tube and stored at -20°C. The protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane at 100 mA for 1.5 h. After blocked by 5% skim milk for 2 h, the membrane was incubated in NF-κB monoclonal antibody (1:2000) at 4°C overnight. Then, the membrane was incubated 30 minutes in goat anti-rabbit secondary antibody at room temperature. Next, the membrane was treated by a developer for 1 min and exposed to observe the result. The film was scanned by Quantity One software and analyzed by the protein image processing system. Each experiment was repeated four times.

Statistical Analysis

All data were presented as mean ± standard deviation (SD). The student's *t*-test was used to compare the differences between the two groups. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data between groups. All data analyses were performed on SPSS11.5 software (SPSS, Inc., Chicago, IL, USA). $p<0.05$ was depicted as statistical significant.

Results

IL-1β mRNA Expression in Rat Hippocampus Tissue

Real Time-PCR was adopted to test IL-1β mRNA expression in rat hippocampus tissue. IL-1β mRNA significantly increased in rat VD model compared with sham group ($p<0.05$). IL-1β antibody injection markedly down-regulated IL-1β mRNA expression in VD model ($p<0.05$) (Figure 1).

IL-1β Content in Rat Serum

ELISA was applied to test IL-1β content in rat serum. IL-1β content markedly elevated in the serum from rat VD model compared with sham group ($p<0.05$). IL-1β monoclonal antibody apparently decreased IL-1β level in rat VD model ($p<0.05$) (Figure 2).

Effects of IL-1β on VD Rat Learning and Memory Abilities

Morris water maze was selected to record escape latency and space probe test. VD rat

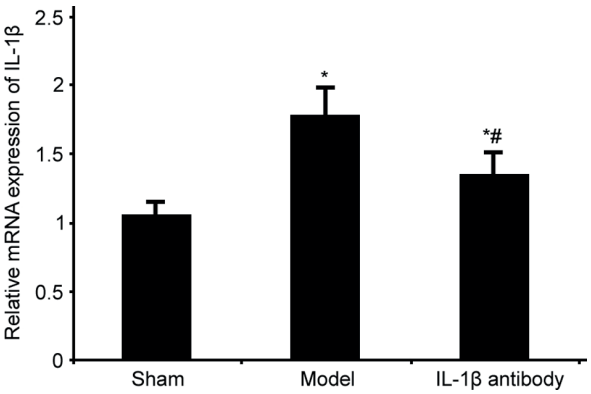

Figure 1. IL-1β mRNA expression in rat hippocampus tissue. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

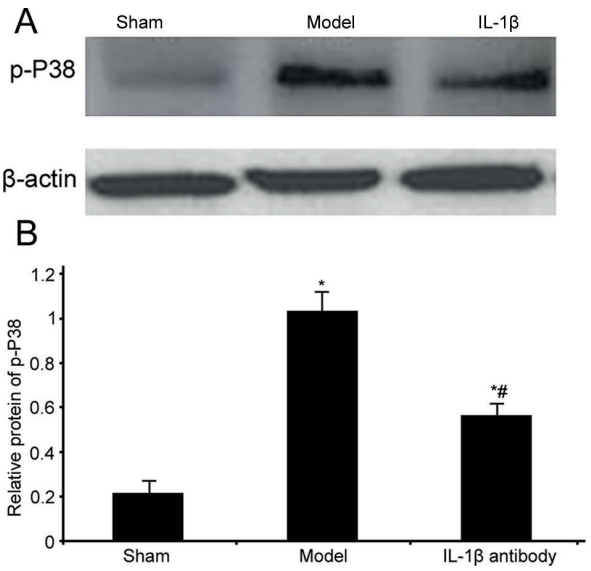

Figure 2. IL-1β expression in rat serum. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

exhibited significantly longer escape latency and reduced times of crossing platform compared with sham group ($p<0.05$). The rat in IL-1β monoclonal group presented shorter escape latency and increased times of crossing platform compared with model group ($p<0.05$) (Figures 3, 4).

Effects of IL-1β on Caspase 3 Activity in Rat Hippocampus Tissue

Caspase 3 activity detection kit was used to determine the effects of IL-1β on caspase 3 activity in rat hippocampus tissue. Caspase 3 activity obviously enhanced in VD rat hippocampus tissue compared with control ($p<0.05$). IL-1β markedly weakened caspase 3 activity in rat hippocampus tissue compared with model group ($p<0.05$) (Figure 5).

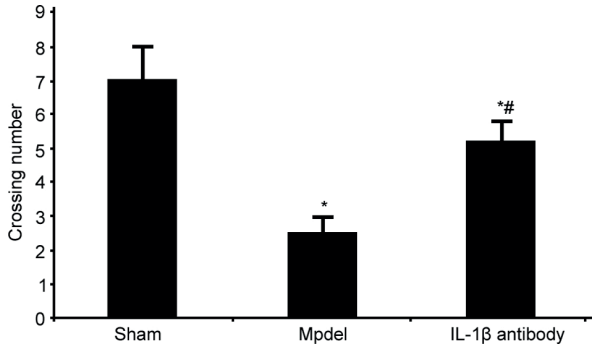

Figure 4. Space probe test record of crossing number. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

Effects of IL-1β on Bcl-2 Expression in Rat Hippocampus Tissue

Western blot was adopted to analyze the impact of IL-1β on Bcl-2 expression in rat hippocampus tissue. Bcl-2 protein significantly decreased in VD rat model compared with sham group ($p<0.05$). It apparently elevated in VD rat treated by IL-1β monoclonal antibody ($p<0.05$) (Figure 6).

Effects of IL-1β on P38MAPK Expression in Rat Hippocampus Tissue

Western blot was adopted to analyze the impact of IL-1β on P38MAPK expression in rat hippocampus tissue. P38 phosphorylation significantly enhanced in VD rat model compared with sham group ($p<0.05$). It apparently decli-

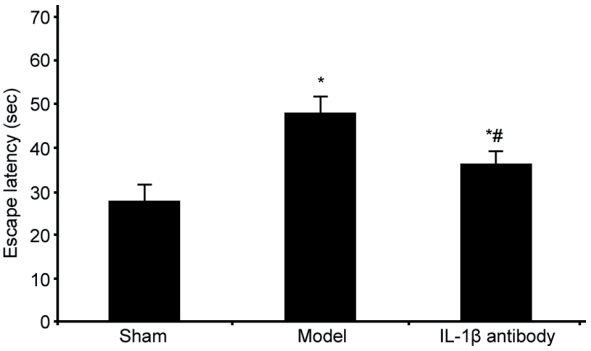

Figure 3. Morris water maze detection of escape latency. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

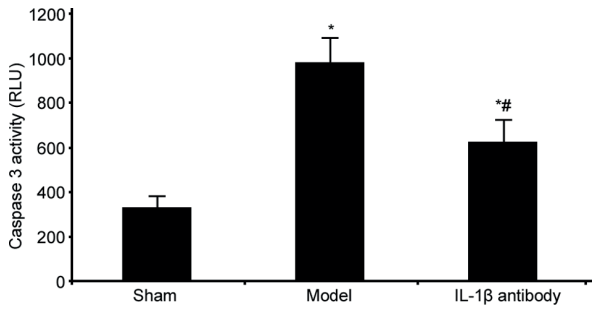

Figure 5. The impact of IL-1β on caspase 3 activity in rat hippocampus tissue. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

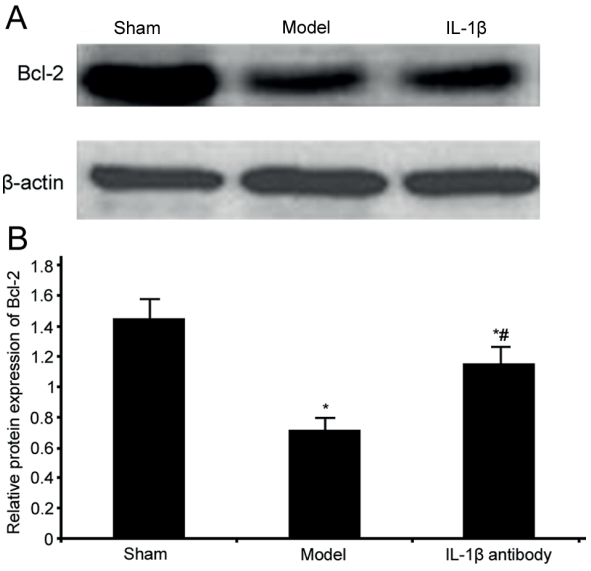

Figure 6. The impact of IL-1β on Bcl-2 expression in rat hippocampus tissue. ***A,*** Western blot detection of Bcl-2 protein expression. ***B,*** Bcl-2 expression analysis. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

ned in VD rat treated by IL-1β monoclonal antibody ($p<0.05$) (Figure 7).

Discussion

A large amount of endothelial cells and neurons are activated in cerebral ischemia, cerebral ischemia-reperfusion injury, and other cerebrovascular diseases, leading to the release of tumor necrosis factor α and IL-1β to induce inflammation[19,20]. During inflammation, IL-1β can facilitate leukocytes adhere to micro-vessel to cause thrombosis. On the other hand, IL-1β can damage neuron and central nervous system directly, resulting in learning and memory hypofunction. IL-1β also can promote leukocytes migration to further damage neuron through accelerating other inflammatory cytokines release and producing inflammatory metabolites, leading to VD occurrence[21,22]. Thus, IL-1β plays a key role in VD, while its specific regulatory mechanism is still unclear.

This study established brain hypoxia and ischemia using bilateral common carotid artery ligation to construct rat VD model[17,23]. Morris water maze was selected to record escape latency and space probe test. VD rat exhibited significantly longer escape latency and reduced times of crossing platform compared with sham group, confirming the successful establishment of VD model. IL-1β monoclonal antibody down-regulated IL-1β expression in hippocampus tissue and serum, thus enhanced learning and memory abilities. P38MAPK is an important member in MAPK family that involves in the regulation of various diseases, including cell cycle, proliferation, inflammation, and stress. Bcl-2 is a type of anti-apoptotic protein that regulates caspase 3. As an initiator of apoptosis, Bcl-2 elevation may inhibit caspase 3 activation to suppress cell apoptosis[24,25]. In this study, P38 MAPK phosphorylation enhanced in VD rat, leading to caspase 3 activation and Bcl-2 reduction. It further affects learning and memory ability of VD rat. It was showed that IL-1β monoclonal antibody significantly reduced IL-1β expression, improved learning ability, attenuated caspase 3 activity, increased Bcl-2 level, and declined p-P38 expression in VD rats.

Conclusions

We observed that IL-1β can delay VD occurrence and development through the p38 MAPK signaling pathway to regulate cell apoptosis and improve learning ability.

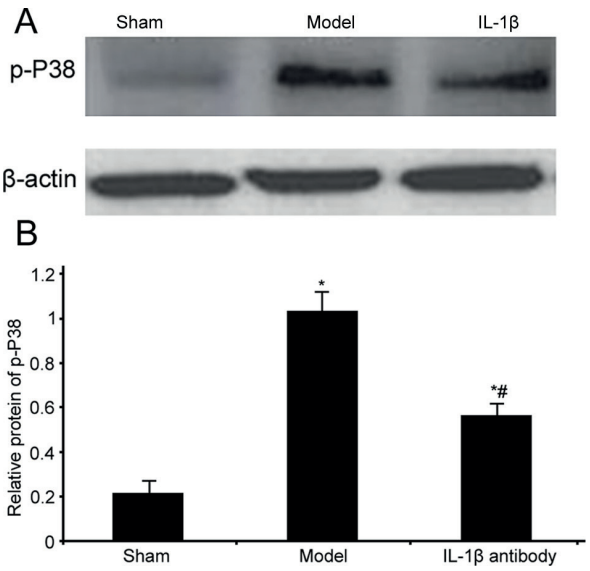

Figure 7. The impact of IL-1β on P38 MAPK expression in rat hippocampus tissue. ***A,*** Western blot detection of P38MAPK protein expression. ***B,*** P38MAPK expression analysis. $^{*}p<0.055$, compared with sham group; $^{\#}p<0.05$, compared with model group.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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