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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 lig and IL-1β group (treated by IL-1β monoclo tibody intracerebroventricular injection on d on model group). Rat learning ability was eva ed by Morris water maze assay. IL-1β expres in brain tissue and peripheral blood as examin by using Real Time-PCR and nked im munosorbent assay (ELISA) pecti Hippocampus apoptosis was det d by ca se 3 activity detection kit. B-cell homa d p38 mitogen-activated ote a tein levels were asse d by W blot assay.

RESULTS: IL-1 pression increased, caspase 3 acti s enhance l-2 level was declined p phosphory tion was elevated in b n tiss d peripheral blood from VD el group co d to sham group ($p<0.05$ 1β monoclonal body significantly re d IL-1 xpression, improved learning abi ttenu caspase 3 activity, increased Bcl- decline -P38 expression in VD rats c to mo group ($p<0.05$).

ONC NS: β can delay VD occur- and ent through the P38-MAPK sig ng path o regulate cell apoptosis and im learning ability.

Key words:
Vascular dementia, IL-1β, P38MAPK, Apoptosis, pase 3, Bcl-2.

Introduction

Cerebrovascular accident and disease, including ischemic stroke and hemorrhage stroke, can induce vascular de ia (VD). It i caused by ischemia an ia, leading t gressive and acquired itive behavior disorder, which seriously affects ling function, memory, and spac . As a comm se of dementia, VD e ts a high morbidity on y second to Alzheimer d ase. It is widel concerned in clinic because of serious influen n quality of life, economic and m pressure society burden[3,4]. Following the e of older population, VD prevention is an im problem needs to be solved in geriatrics and related disciplines[5,6]. High incidence rate osclerosis, hypertension, and cardiovascular d rovascular 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 monoclon tibody, Bcl-2 monoclonal antibody, and g ti-rabbit horseradish peroxidase (HRP) la d IgG secondary antibody were provided by Signaling Technology (Beverly, MA, USA). R extraction kit and reverse trans kit we purchased from ABI (Foster C A). ABI 7500 Real Time-PCR amp was d ed from ABI (Foster City, CA, US icros instrument was purch d apparatus factory hou,). Multi-Parameter Monitor al physio monitor, electroencepha EG) record nd YC-2 stimulator we purcha om Yuyanbio (Shanghai, Chi DNA ampli as obtained from PE Ge pplied Biosystem ode: Gene Amp PCR tem 24 Foster, CA, USA). Labsystem Ve 3.1 ro-plate reader was provided by B borato (Hercules, CA, USA). r rea wer chased from Sangon Bio- logy Shanghai, China).

Experimental Animal Grouping and Treatment

The healthy male rats were randomly divided three groups, including sham group, VD mo- up 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 h te abdominal injection and fixed on ster paratus. Then, the neck skin was di cted and the incision was made on the nec court line. The muscle and connective tissues parated to isolate the bilateral comm carotid for ligation. The vagus nerve protected damage. Rat breathes a eart ra were o ved. 10^4 U gentamyc as us r three days after surgery to pr en The ra the sham group rec the reatm ithout bilateral comm carotid art

Morris Water Maze Test

Navigation test an probe test in the Morris w ze test were ied[18]. The time from e water to reach under ater platform was re- ed as escape ency. Rat swimming time and imes of cros g platform to search platform w 120 s wer corded to test rat learning and me bili

Sample Collection

al of 2 ml blood was extracted from the rat in 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	TAATGATAGGTGACCCCTGC
IL-1β	CCCTGCCCTGTATTACAATC	GATGGTATTTATGATATC

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 adde radioimmunoprecipitation assay (RIPA) c ning protease inhibitor and cracked on ic 15-30 min. Next, the tissues were treated by trasound at 5 s for 4 times and c ed 15 m nutes at 10000 ×g. The protei s t ferred to a new Eppendorf (Ep) tub d store -20°C. The protein was separat 10% decyl sulfate polyacr mid trophic s (SDS-PAGE) and sferred VDF membrane at 100 mA 5 h. After ed by 5% skim milk for embrane w ncubated in NF-κB mo clonal ody (1:2000) at 4°C overnight en, the me e was incubated 30 mi in goat anti-ra econdary antibody oom t erature. Next, the membrane wa ted h developer for 1 min and exposed to the re The film was scanned Quan One s are and analyzed by the p ima ssing system. Each experiment was repe four times.

Statistical Analysis

All data were presented as mean ± standard viation (SD). The student's *t*-test was used to mpare 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 (SS, Inc cago, IL, USA). $p<0.05$ was de d as statis gnificant.

Results

IL-1β mRN ession in Hippoca us

Real Time-PCR adopted to test IL-1β mR pression in ippocampus tissue. IL mRNA significantly increased in rat VD el compared ith sham group ($p<0.05$). IL- ntibody inje n markedly down-regulated IL mRNA ex ssion in VD model ($p<0.05$) (Fig

IL-1β Content in Rat Serum

SA was applied to test IL-1β content in rat 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

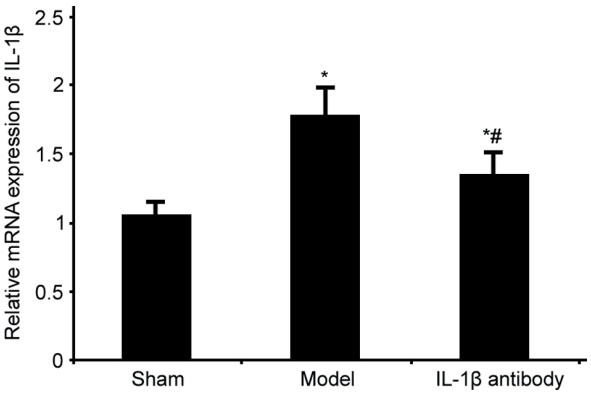

Figure 1. IL-1β mRNA expression in rat hippocampus tissue. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

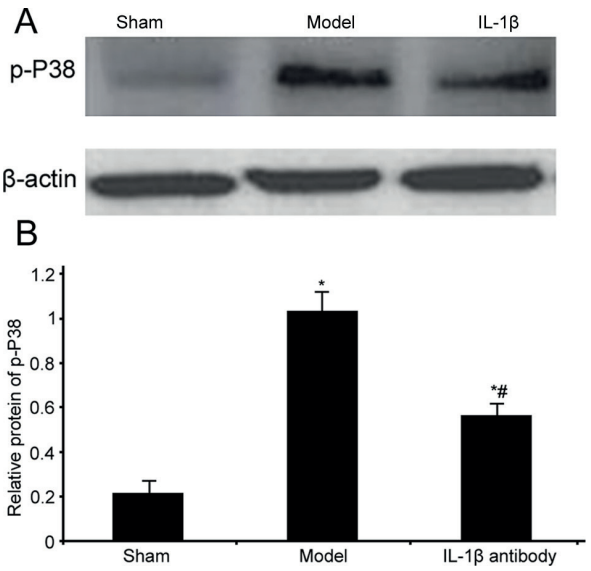

Figure 2. IL-1β expression in rat serum. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

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

Effects of IL-1β on Caspa ity in Rat Hippocampus Tiss

Caspase 3 activity de n kit determine the effects IL- pase 3 vity in rat hippocam tissue. ase 3 activity obviously enh d in VD ra ocampus tissue compar ntrol ($p<$). IL-1β

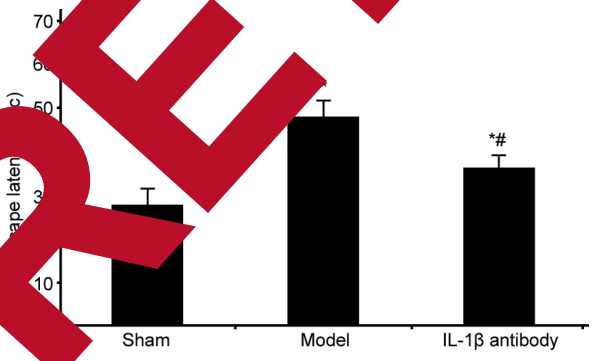

Figure 3. Morris water maze detection of escape latency. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

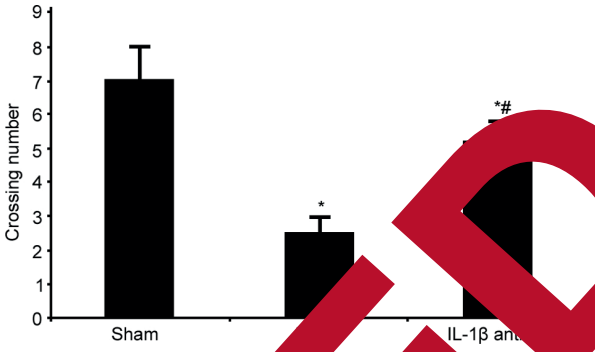

Figure 4. Space probe ecord ossing numb r. $^{*}p<0.05$, compared wi <0.05, pared with model group.

markedly aken aspase 3 ctivity in rat hippocampus tissue ared with model group ($p<$ igure 5).

ects of IL-1β n Bcl-2 Expression at Hippoc ous Tissue

tern blot adopted to analyze the impac 1β cl-2 expression in rat hippocampu Bcl-2 protein significantly deeased in VD rat model compared with sham ($p<0.05$). It apparently elevated in VD rat ea 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-

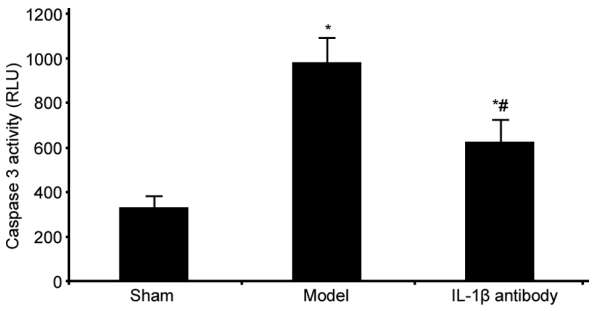

Figure 5. The impact of IL-1β on caspase 3 activity in rat hippocampus tissue. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

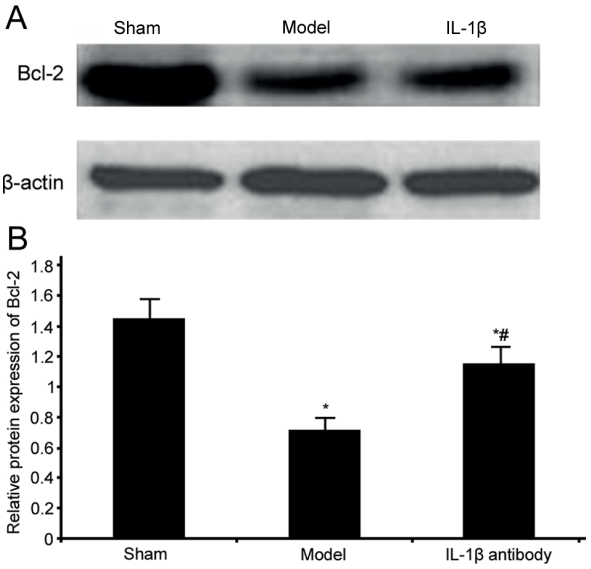

Figure 6. The impact of IL-1β on Bcl-2 expression in rat hippocampus tissue. ***A,*** Western blot detection of Bcl-2 protein expression. ***B,*** Bcl-2 expression analysis. $^{*}p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with model group.

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

Discussion

A large amount of endoth... and neu-rons are activated in cereb... schem... cerebral ischemia-reperfusion inj... nd ot... vascular diseases, lea... g t... se of t... r necrosis factor α a... L-1β to ... e inflamma-tion[19,20]. During i... mmation, I... an facili-tate leukocyte ... micro-ves... to cause thrombosis. O... the oth... nd, IL-1β can dama-ge neuro... d central ne... system directly, resulti... learning and me... y hypofunction. IL-1... so can ... omote leukocytes migration to ... r da... e neuron through accelerating other ... atory c... kines release and pro-...ing i... mato... etabolites, leading to VD ... ence ..., IL-1β plays a key role in VD ... hile its ... ific regulatory mechanism is sti... her.

... established brain hypoxia and ...chemia using bilateral common carotid artery ...ion to construct rat VD model[17,23]. Morris w... maze was selected to record escape laten-cy and space probe test. VD rat exhibited signi-ficantly 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-regu-lated IL-1β expression in hippocampus tissue and serum, thus enhanced learning and m... y abilities. P38MAPK is an important ... MAPK family that involves in the ...iation o... various diseases, including cell ... proliferа-tion, inflammation, and stress. B... a type of anti-apoptotic protein th... egulat... pase 3. As an initiator of apo...s, Bcl-2 e... may inhibit caspase 3 a...ation t...uppres... apoptosis[24,25]. In thi...dy, P...MAPK pho-sphorylation enha...ed ...D..., leadi... ca-spase 3 activati...d Bc...uction ...urther affects learni...d memor...li... VD rat. It was sho... IL-1β mon...al antibody significa...redu... IL-1β expression, impro-ved learning ability, ...uated caspase 3 acti-vity ...sed Bcl-2 l... and declined p-P38 e...ssion in VD rats.

...nclusions

We ... that IL-1β can delay VD occur-...ence and development through the p38 MAPK ...ing pathway to regulate cell apoptosis ...rove learning ability.

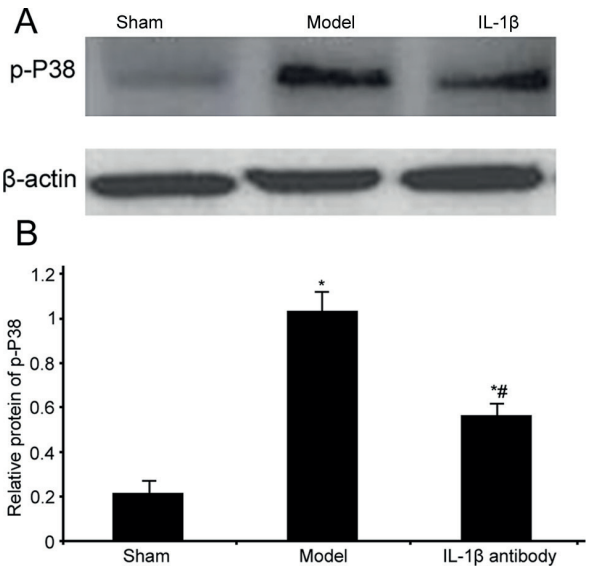

Figure 7. The impact of IL-1β on P38 MAPK expression in rat hippocampus tissue. ***A,*** Western blot detection of P38MAPK protein expression. ***B,*** P38MAPK expression analysis. $^{*}p<0.055$, compared with sham group; $^{\#}p<0.05$, compared with model group.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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