Am J Transl Res 2021;13(4):2213-2223
www.ajtr.org /ISSN:1943-8141/AJTR0126019

Original Article

Rehabilitation training inhibits neuronal apoptosis by down-regulation of TLR4/MyD88 signaling pathway in mice with cerebral ischemic stroke

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Received November 10, 2020; Accepted February 9, 2021; Epub April 15, 2021; Published April 30, 2021

Abstract: Objective: To investigate the role of rehabilitation training and TLR4/MyD88 signaling pathway on neuronal apoptosis in mice with cerebral ischemic stroke. Methods: Mice were randomized into six groups, which were normal group (healthy mice, n=20), control group (sham surgery, n=20), model group (middle cerebral artery occlusion (MCAO) model, n=20), training (MCAO model, continuous rehabilitation training for 4 weeks, n=20), TAK-242 group (MCAO model, TL R4 inhibitor TAK-242, n=20), and TAK-242 + Training group (MCAO model, TLR4 inhibitor TAK-242 + rehabilitation training, n=20). Results: Neurobehavioral assessment was performed, and cerebral infarction area of mice was detected by triphenyl tetrazolium chloride staining. Compared with the normal group, no significant differences in all indicators were found in the control group (all $P>0.05$), while the other groups had higher neurological function scores, cerebral infarction area, neuronal apoptosis rate, increased expressions of TLR4, MyD88, Bax, NF-κB, TNF-α, Caspase-3, IL-1βA and decreased mRNA and protein expressions of Bcl-2 (all $P<0.05$). Conclusion: Rehabilitation training can effectively reduce the apoptosis of hippocampal neurons in mice with ischemic stroke by inhibiting the TLR4/MyD88 signaling pathway.

Keywords: TLR4/MyD88 signaling pathway, rehabilitation training, ischemic stroke, hippocampal neuron, apoptosis

Introduction

The proportion of patients with ischemic stroke among patients with stroke has been increasing. With high mortality, ischemic stroke, whose pathogenesis of ischemic stroke has not been fully elucidated, severely affects the life and health of middle-aged and elderly people, [1-4]. Therefore, it is greatly significant to explore the pathogenesis and treatment of ischemic stroke.

At present, rehabilitation training, as the main treatment strategy of ischemic stroke its clinical efficacy has been recognized worldwide [5]. Studies have demonstrated that rehabilitation training can reduce inflammatory injury, thereby inhibiting or delaying the apoptosis of ischemic cerebral neurons and reducing the degree of cerebral ischemia [6-9]. However, due to the unclear specific mechanism of rehabilitation training, wide application of rehabilitation therapy is restricted.

Toll like receptor (TLR) is a main receptor for the natural immune system to recognize pathogens, which plays an essential role in the natural immune response. It recognizes pathogens and initiates natural immunity in the early stages of pathogen invasion, triggers inflammatory responses, and serves a role of anti-pathogenic microorganisms [10-14]. It is currently believed that the signal transduction pathway of TLR4 can be roughly divided into MyD88 (mainly exist in the brain tissues) and non-MyD88 signaling pathways. Recently, many studies have reported that TLR4/MyD88 signaling pathway has an essential role in cerebral inflammatory injury. TLR4 mainly expresses in hippocampal microglia and participates in inflammatory factor

release and neuronal cell apoptosis. As an adaptor of TLR4, MyD88 leads to secretion of inflammatory cytokines and activates the Caspase-3. Inflammatory injury is involved in brain injury, and TLR4-mediated MyD88 signal transduction pathway is a key signal transduction pathway for brain injury [15-21]. However, it has not been confirmed whether TLR4/MyD88 signaling pathway participates in the anti-apoptotic effects of rehabilitation training on neurons of mice with ischemic stroke, thereby affecting the amelioration of ischemic stroke.

Therefore, in this study, mice treated by middle cerebral artery occlusion (MCAO) were given TLR4/MyD88 signaling pathway inhibitor, rehabilitation training only or the combination of both, to explore whether TLR4/MyD88 signaling pathway participated in the anti-apoptotic effect of rehabilitation training on neurons of mice with ischemic stroke, with hope to provide objective evidence for the mechanism of rehabilitation training on the remodeling of cerebral function in mice with cerebral infarction.

Material and methods

Animals

One hundred and twenty healthy male 8-week specific-pathogen-free BALB/c mice t were selected (21.18±2.94 g, provided by Animal Center of Guangxi Medical University, permit NO. of laboratory mice: SCXK (Gui) 2016-0036). Eighty mice were picked randomly to establish MCAO model, and the rest were divided into the normal group (n=20) and the control group (n=20). They were fed with a normal diet in our laboratory before modeling.

MCAO model

Suture occlusion method was used to establish MCAO model with the reference of Odilo's study [22]. Inhalation of 1.5-2% isoflurane (Shanghai Yuyan Scientific Instrument Co., Ltd., China) was given to anesthetize mice. Left common carotid artery (CCA) and external carotid artery were separated and ligated. The distal end of the internal carotid artery (ICA) was closed with a vessel clip. An incision was made near the crotch of CCA. After the vessel clip was withdrawn, a 7.0 nylon suture with silicone (10 mm) was inserted from CCA into ICA. The middle cerebral artery blood flow was occluded and the spare line was tightened. After 60 min, the nylon suture was removed. The mouse was maintained at 37°C during surgery, and then the skin was sutured. Cerebral blood flow was monitored with a 0.5 mm fiber optic probe. Left middle cerebral artery was located at 6mm lateral to and 2 mm behind the fontanel. The surgical procedure in the control group was similar to create the MCAO model, however, the occluding suture was immediately withdrawn after inserted, followed by skin suture. During the surgery, the anus temperature of the mouse was measured and maintained at 37.0±0.5°C until the mouse recovered naturally. The success of model establishment was defined as the blood flow value measured by the laser Doppler flowmetry (FLO-C1; Omegaflo, Tokyo, Japan) was reduced to less than 25%.

Grouping

The mice were randomized into six groups, which were normal group (healthy mice, n=20), control group (sham surgery, n=20), model group (MCAO model, n=20), training group (MCAO model, continuous rehabilitation training for 4 weeks, n=20), TAK-242 group (MCAO model, TLR4 inhibitor TAK-242, n=20), and TAK-242 + Training group (MCAO model, TLR4 inhibitor TAK-242 + rehabilitation training, n=20). After the mice in the TAK-242 and TAK-242 + training groups woke up, they were intravenously injected with 5,000 pmol/l TAK-242 (HY-11109, MCE, USA) slowly at a flow velocity of 0.001 l/h for 2 h, once per day for 2 weeks [23]. The mice in the training and TAK-242 + training groups received rehabilitation training after they woke up [24]. The rehabilitation exercise consisted of a roller training, a rotating bar training, and a balance beam training. In the roller training, the mice were trained with self-made roller-type mesh training equipment (100.0 cm in length, 60.0 cm in diameter). The equipment was divided into four grids to train four mice at the same time. There was a fixed frame under the base and one handle at the end. It was used at 5 r/min, 10 min/time and twice/day in hand crank to train the mice to grasp, walk and rotate. In the rotating bar training, one rotating bar (150.0 cm in length, 4.5 cm in diameter) was fixed at the middle point on the 3 r/min rotator, with an alternately clockwise and counterclockwise rotation (10 min/time, twice/day). Mice should move from one end of the bar to the other, to gain dynamic balance ability. In the balance beam training, the balance beam was 170.0

cm in length and 2.0 cm in width. There was a 7.0-cm-high bracket under the balance beam. The mice crawled on the balance beam after it was laid flat at 15 cm from the ground to gain balance ability (10 min/time, and twice/day). All training was performed continuously for 4 weeks.

Ethical approval and consent to participate

The study gained the approval of the Ethics Committee of Shaoxing Hospital of Traditional Chinese Medicine.

Neurobehavioral assessment

After the rehabilitation training, neurobehavioral assessment of mice was carried out according to the neurological disability status scale from Rodolfo [25]. No significant changes in neural behavior were scored as 0. Reduced responsiveness and a slight decrease in the number of activities were scored as 2 points. A moderate decrease in activity, biased to one side, disordered gait, and decreased muscle ability were scored as 4 points. Significantly reduced activities, flexed forelimbs, and lost behavior of mild exercises such as convulsions and circling were scored as 6 points. Difficulty breathing, severely reduced activities, and severe dyskinesia were scored as 8 points. Died during the process were recorded as 10 points. If the behavior of the mice was close to the scale mentioned above, the behavior of the mice was then rated as that score minus one.

Triphenyl tetrazolium chloride (TTC) staining

After the rehabilitation training, the head of five mice were removed from the body and the brain tissue was taken out. The structural integrity of the brain should be maintained during the surgery. Normal saline was used to clean the blood, and the brain tissue was quickly frozen at -20°C. After 20 min, the brain tissue was taken out to make a coronal section with a thickness of 2.0 mm. The prepared sections were immersed in a pre-configured 1% TTC solution (Shanghai Yansheng Biochemical Reagent Co., Ltd., China) and incubated at 37°C under constant temperature and illumination, and the sections were inverted to ensure uniform staining. The normal area was dyed to uniform bright red, the infarcted portion was white and no longer colored, and the process was for about 15 minutes. The stained brain sections were fixed in 10% formalin. After that, the brain sections were taken out and carefully blotted to dry the water on the surface. The digital camera was used to take a coronal slice in the order of the front and back of the brain, and images were imported to a computer. Image J processing software was used to calculate the proportion of cerebral infarction area of mice in each group.

Flow cytometry

After rehabilitation training, five mice in each group were decapitated to obtain the hippocampus tissues. The tissues were cut, homogenized, and water-bathed at 37°C for 6 min. Pancreatin (3 mL, 0.25%, Sigma-Aldrich, USA) was then added, and after being gently pipetted up and down, the supernatant was collected and filtered using a 220 mesh strainer. The filtered solution was centrifuged at 3,200 g for 10 min followed by supernatant discard and cell collect. The cell suspension in each group was prepared using serum-free DMEM and digested by EDTA-free pancreatin. Subsequently, the cells were collected in flow cytometry tubes and centrifuged. After supernatant discard, the cells were washed with cold PBS for three times and centrifuged again followed by discarding the supernatant. According to the instruction of Annexin-V-FITC apoptosis detection kit (APOAF, Sigma, USA), Annexin-V-FITC, PI, HEPES buffer solutions at 1:2:50 were used to prepare Annexin-V-FITC/PI dye liquor. Every 1×10^6 cells were re-suspended in 100 μL dye liquor, mixed by shaking, and then incubated for 15 min at room temperature. Afterward, 1 ml HEPES buffer was added and mixed by shaking. The FITC and PI fluorescence was detected at 525 nm and 620 nm respectively to examine cell apoptosis. Apoptosis rate = number of apoptotic cells/(number of apoptotic cells + number of normal cells).

qRT-PCR

Hippocampal tissues of five mice in each group were collected for making homogenate. Liquid nitrogen was added to the homogenate and ground into fine powder. Total RNA from the homogenate was extracted using TRIzol (Invitrogen, New York, USA) and checked for its purity and concentration. Then, RNAs were reversely transcribed into cDNAs according to the instruction of Prime Script TMRT Reagent Kit (TaKaRa, Dalian, China). The total reaction sys-

Table 1. qRT-PCR primer sequences

Genes	qRT-PCR primer sequences (5'-3')
TLR4	Forward: ATGGCATGGCTTACACCACC
	Reverse: GAGGCCAATTTTGTCTCCACA
MyD88	Forward: TCATGTTCTCCATACCCTTGGT
	Reverse: AAACTGCGAGTGGGGTCAG
NF-κB	Forward: ATCAGGCGACAGGTGAACAG
	Reverse: GGCCACAGCAGTTCTCGAA
TNF-α	Forward: CAGGCGGTGCCTATGTCTC
	Reverse: CGATCACCCCGAAGTTCAGTAG
IL-1β	Forward: GAAATGCCACCTTTTGACAGTG
	Reverse: TGGATGCTCTCATCAGGACAG
Bcl-2	Forward: GTCGCTACCGTCGTGACTTC
	Reverse: CAGACATGCACCTACCCAGC
Caspase-3	Forward: ATGGAGAACAACAAAACCTCAGT
	Reverse: TTGCTCCCATGTATGGTCTTTAC
Bax	Forward: TGAAGACAGGGGCCTTTTTG
	Reverse: AATTCGCCGGAGACACTCG
GAPDH	Forward: AGGTCGGTGTGAACGGATTTG
	Reverse: GGGGTCGTTGATGGCAACA

Note: TLR, toll-like receptor; NF, nuclear transcription factor; TNF, tumor necrosis factor; IL, interleukin.

tem was 25 μL. The reversed cDNA was diluted with 65 μL of DEPC and fully mixed. cDNAs were used for polymerase chain reaction (PCR) amplification. Quantitative real-time PCR (qRT-PCR) was performed according to the instruction of SYBR® Premix Ex Taq™ II (TaKaRa, Dalian, China). The total reaction system was 50 μL including 25 μL (2×) SYBR® Premix Ex Taq™ II, 2 μL forward primer, 2 μL reverse primer, 1 μl ROX Reference Dye (50×), 4 μL cDNA template, 16 μl deionized water. qRT-PCR was carried out using a quantitative PCR amplifier (PRISM® 7300, ABI, USA). The reaction conditions were 40 circles of pre-denaturation (95°C, 4 min), denaturation (94°C, 30 s), annealing (58°C, 30 s), and extension (72°C, 1 min), followed by extension (72°C, 7 min) after the last cycle. GAPDH was an internal control. Their primer sequences, synthesized by Bojie (Wuhan, China), are listed in **Table 1**. The ratio expression of target gene was expressed by $2^{-\Delta\Delta Ct}$. $\Delta\Delta CT=\Delta Ct_{experimental\ group} - \Delta Ct_{control\ group}$. $\Delta Ct=Ct_{target\ gene} - Ct_{GAPDH}$. Ct represents the number of amplification cycles for the fluorescence intensity to reach the threshold after the sample is amplified by qPCR. At this point, the amplification is in logarithmic growth.

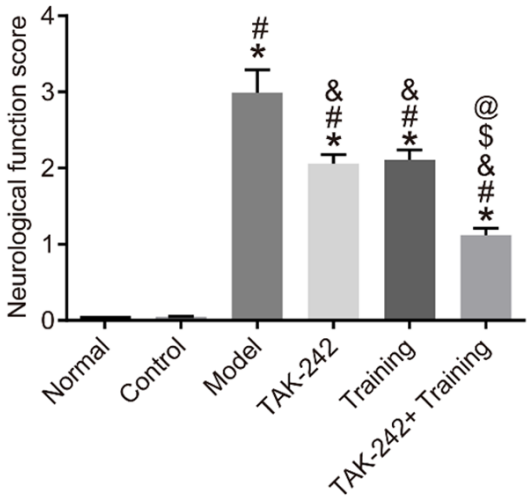

Figure 1. Neurobehavioral assessment outcome. Compared with the normal group, *P<0.05; compared with the control group, #P<0.05; compared with the model group, &P<0.05; compared with the TAK-242 group, $P<0.05; compared with the Training group, @P<0.05.

Western blot

Hippocampal tissues of five mice in each group were collected for making homogenate. Liquid nitrogen was added to the homogenate and ground into fine powder, and the lysate (Sigma, USA) was then added. The samples were centrifuged at 12,000×g for 15 min at 4°C to collect the supernatant. BCA protein assay was used for protein quantification, and SDS-PAGE (10%) was prepared. The protein sample was mixed with loading buffer and placed in a boiling bath at 100°C for 5 min. SDS-PAGE was conducted after the sample was placed in an icy bath and centrifuged. Proteins were transferred to polyvinylidene fluoride membrane, and the membrane was blocked with 5% skim milk for 2 h at 4°C overnight. The membrane was added with primary anti-rabbit antibodies including TLR4 (1:500, Abcam, USA), MyD88 (1:1,000, Abcam, USA), nuclear transcription factor-κB (NF-Kb, 1:2,000, Abcam, USA), tumor necrosis factor-α (TNF-α, 1:2,000, Abcam, USA), interleukin-1β (IL-1β, 1:1,000, Abcam, USA), Bcl-2 (1:2,000, Abcam, USA), Caspase-3 (1:500, Abcam, USA), Bax (1:1,000, Abcam, USA) and incubated overnight. The samples were washed in PBS for three times (5 min per wash) and incubated with secondary antibody goat anti-rabbit IgG (1:1,000, Abcam, USA) for 1 h at 37°C before being washed in PBS for three times (5 min per

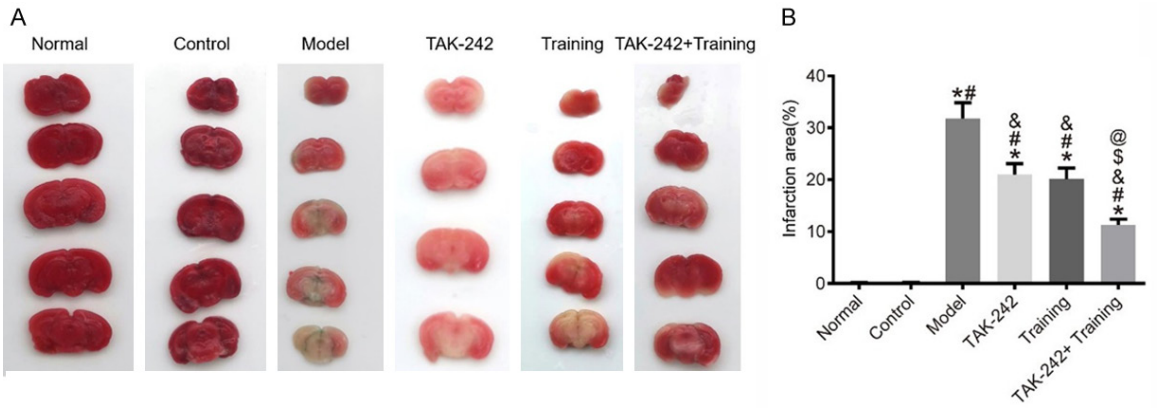

Figure 2. Cerebral infarction area in each group. A: TTC-stained sections; B: Cerebral infarction area. Compared with the normal group, *P<0.05; compared with the control group, #P<0.05; compared with the model group, &P<0.05; compared with the TAK-242 group, $P<0.05; compared with the Training group, @P<0.05.

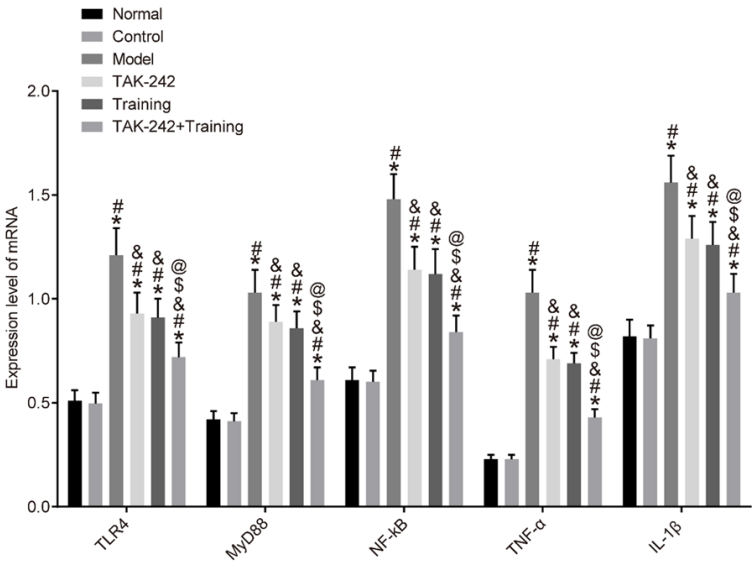

Figure 3. Expressions of TLR4, MyD88, NF-κB, TNF-α and IL-1β mRNAs in the TLR4/MyD88 signaling pathway detected by qRT-PCR. Compared with the normal group, *P<0.05; compared with the control group, #P<0.05; compared with the model group, &P<0.05; compared with the TAK-242 group, $P<0.05; compared with the Training group, @P<0.05. TLR: toll-like receptor.

wash). The membrane was covered with ECL solution (36208ES60, Shanghai Shengsheng Biological Technology Co., Ltd., Shanghai, China), exposed, developed and fixed in the dark to observe the experimental outcome. Relative protein level = grayscale value of the target band/grayscale value of the GAPDH internal control.

Statistical analysis

SPSS 21.0 software (SPSS Inc., Chicago, USA) was used for statistical analysis and GraphPad Prism 7 was used to draw figures. Measurement data were expressed as mean ± sd. One-way analysis of variance and post-hoc Bonferroni pairwise comparison were applied to compare among groups. P< 0.05 was defined as a statistically significant difference.

Results

Neurobehavioral assessment

Neurobehaviors of mice in each group were scored (**Figure 1**). Compared with the normal group, no significant difference was found in neurological function scores in the control group (P>0.05), while significantly increased in other groups (all P< 0.05). Compared with the model group, TAK-242, and TAK-242 + training groups had lower neurological function scores (all P<0.05). Compared with the TAK-242 group, the neurological function score was lower in the TAK-242 + training group (P<0.05).

Cerebral infarction area

Cerebral infarction area in each group was detected by TTC staining (**Figure 2**). Compared with the normal group, no significant difference was found in cerebral infarction area in the control group (P>0.05), while was significantly increased in the other groups. Compared with

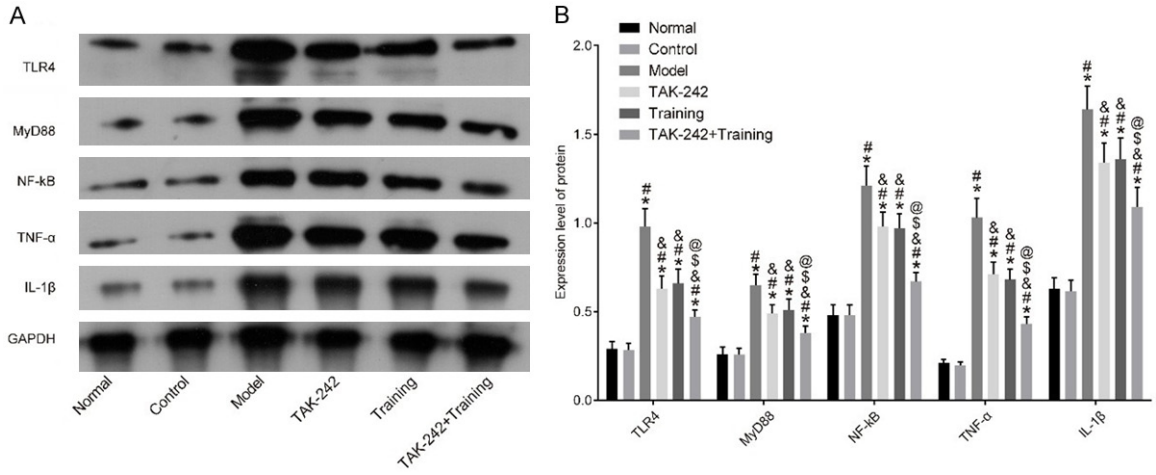

Figure 4. Protein expressions of TLR4, MyD88, NF-κB, TNF-α, and IL-1β in TLR4/MyD88 signaling pathway detected by Western blot. A: Protein bands; B: Protein expressions. Compared with the normal group, *P<0.05; compared with the control group, $^{\#}$P<0.05; compared with the model group, $^{\&}$P<0.05; compared with the TAK-242 group, $^{\$}$P<0.05; compared with the Training group, $^{@}$P<0.05. TLR, toll-like receptor.

the model group, the cerebral infarction area was lower in the training, TAK-242, and TAK-242 + training groups (all P<0.05). The TAK-242 + training group had lower cerebral infarction area than the TAK-242 group (P<0.05).

mRNA expressions of TLR4, MyD88, NF-κB, TNF-α and IL-1β by qRT-PCR

qRT-PCR was used to detect the expression of TLR4, MyD88, NF-κB, TNF-α, and IL-1β mRNAs in the TLR4/MyD88 signaling pathway of mice in each group (**Figure 3**). Compared with the normal group, the mRNA expressions of the mentioned indicators were higher in the other groups (all P<0.05) except for the control group (all P>0.05). Compared with the model group, the mRNA expressions of the mentioned indicators were lower in the training, TAK-242, and TAK-242 + training groups (all P<0.05). Compared with the TAK-242 group, the mRNA expressions of TLR4, MyD88, NF-κB, TNF-α, and IL-1β were lower in the TAK-242 + training group (all P<0.05).

Protein expressions of TLR4, MyD88, NF-κB, TNF-α, and IL-1β by Western blot

Western blot was applied to detect the protein expressions of TLR4, MyD88, NF-κB, TNF-α, and IL-1β in TLR4/MyD88 signaling pathway (**Figure 4**). Compared with the normal group, the protein expressions of the mentioned indicators were higher in the other groups (P<0.05) except for the control group (P>0.05). Compared with the model group, the protein expressions of the mentioned indicators were lower in the training, TAK-242, and TAK-242 + training groups (all P<0.05). The TAK-242 + training group had lower protein expressions of TLR4, MyD88, NF-κB, TNF-α, and IL-1β than the TAK-242 group (all P<0.05).

Hippocampal neuronal apoptosis by flow cytometry

Flow cytometry was applied for detection of hippocampal neuronal apoptosis (**Figure 5**). Compared with the normal group, the hippocampal neuronal apoptosis rate was higher in the other groups (all P<0.05) except for the control group (P>0.05). The training, TAK-242, and TAK-242 + training groups had lower hippocampal neuronal apoptosis rate than the model group (all P<0.05). Compared with the TAK-242 group, the apoptosis rate was lower in the TAK-242 + training group (P<0.05).

mRNA expressions of Bcl-2, Caspase-3, and Bax by qRT-PCR

qRT-PCR was used to detect the expressions of apoptosis-associated factors, Bcl-2, Caspase-3, and Bax mRNAs, in mice' hippocampal neurons (**Figure 6**). Compared with the normal group, the Bcl-2 mRNA expression was lower, whereas the mRNA expressions of Caspase-3 and Bax were higher in the other groups (all

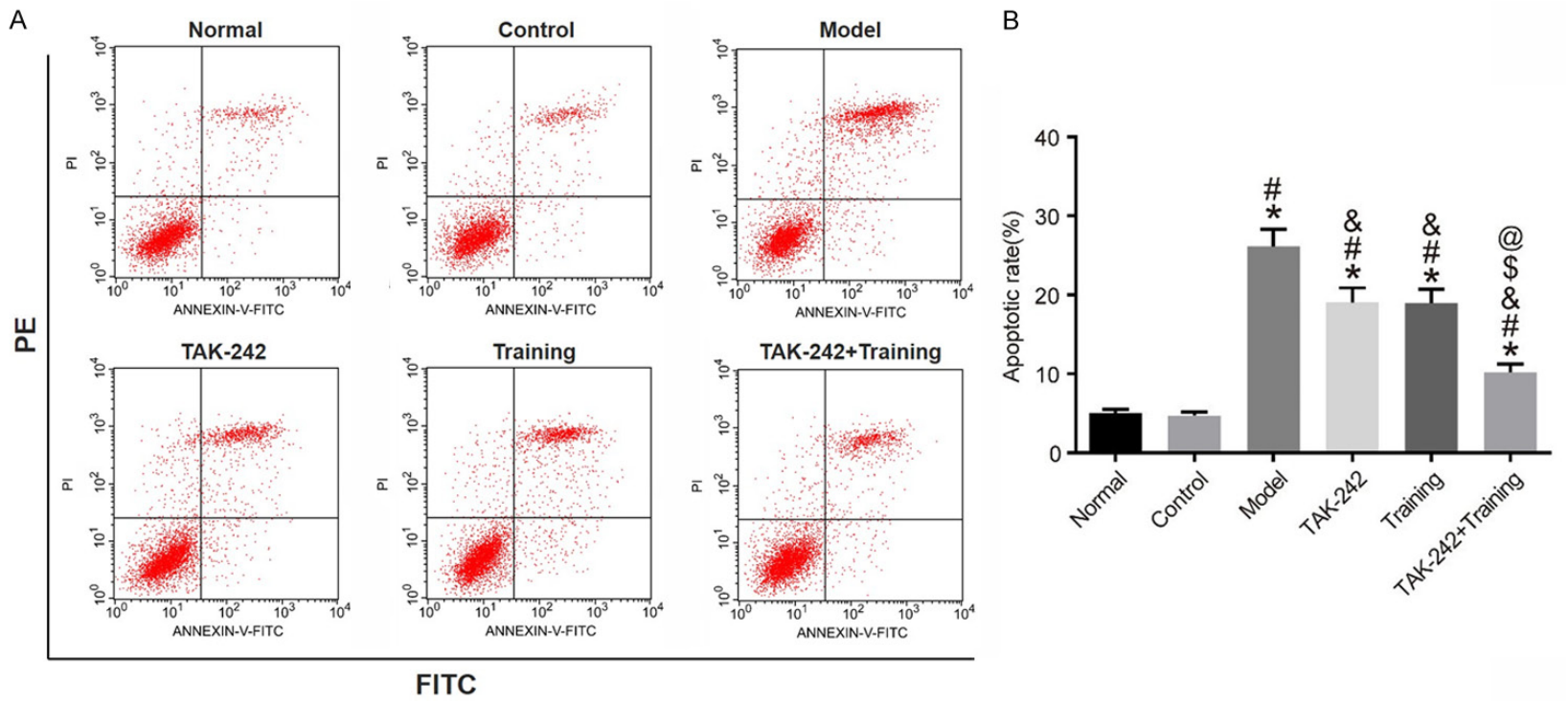

Figure 5. Hippocampal neuronal apoptosis detected by flow cytometry. A: Flow cytometry image; B: Hippocampal neuronal apoptosis rate. Compared with the normal group, $^{*}P<0.05$; compared with the control group, $^{\#}P<0.05$; compared with the model group, $^{\&}P<0.05$; compared with the TAK-242 group, $^{\$}P<0.05$; compared with the Training group, $^{@}P<0.05$.

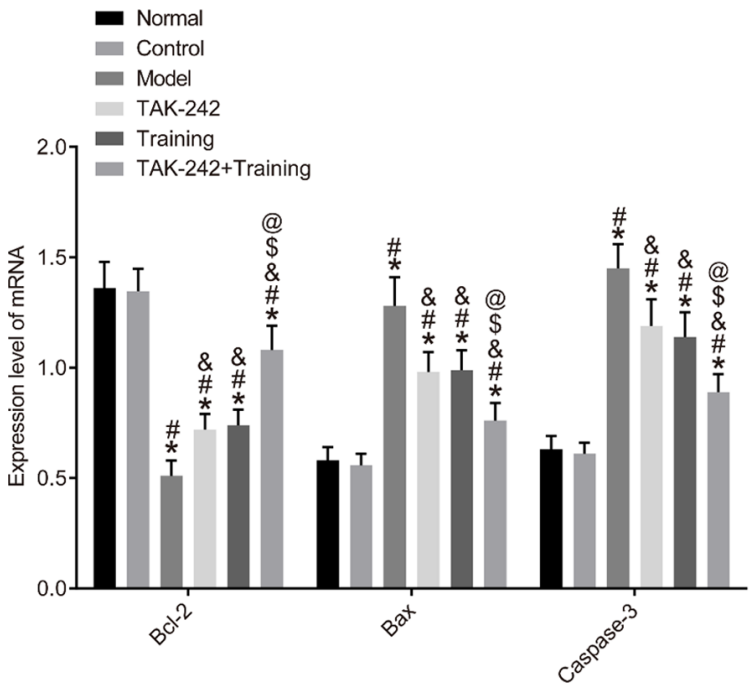

Figure 6. Expressions of Bcl-2, Bax, and Caspase-3 mRNAs detected by qRT-PCR. Compared with the normal group, *P<0.05; compared with the control group, #P<0.05; compared with the model group, &P<0.05; compared with the TAK-242 group, $P<0.05; compared with the Training group, @P<0.05.

P<0.05) except for the control group (P>0.05). Compared with the model group, the Bcl-2 mRNA expression was higher, while the mRNA expression level of Caspase-3 and Bax were lower in the training, TAK-242, and TAK-242 + training groups (all P<0.05). Compared with the TAK-242 group, the Bcl-2 mRNA expression was higher, while the expression levels of Caspase-3 and Bax mRNAs were lower in the TAK-242 + training group (all P<0.05).

Protein expressions of Bcl-2, Caspase-3, and Bax by Western blot

Western blot was used to detect the protein expressions of Bcl-2, Caspase-3, and Bax (**Figure 7**). Compared with the normal group, the Bcl-2 protein expression was lower, whereas the protein expression levels of Caspase-3 and Bax were higher in the other groups (all P<0.05) except for control group (P>0.05). Compared with the model group, the Bcl-2 protein expression level was higher, while the protein expression levels of Caspase-3 and Bax were lower in the training, TAK-242, and TAK-242 + training groups (all P<0.05). Compared with the TAK-242 group, Bcl-2 protein expression level was higher, while Caspase-3 and Bax protein expression levels were lower in the TAK-242 + training group (all P<0.05).

Discussion

Ischemic stroke has been severely threatening the life and health of middle-aged and elderly patients. Studying on the treatment of ischemic stroke and related mechanisms has far-reaching significance for improving patient's quality of life [26-29]. In this study, a MCAO model was created to simulate ischemic stroke in humans and explore the related mechanisms.

Rehabilitation training for patients with ischemic stroke has now been widely recognized. It is known that rehabilitation training can improve cerebral ischemia by inhibiting or delaying the apoptosis of nerve cells after cerebral ischemia [30-32]. At the same time, studies have revealed that TLR4/MyD88 signaling pathway is an essential role in cerebral inflammatory injury involved in brain injury [33, 34]. NF-κB, TNF-α and IL-1β are inflammatory factors which are involved in various diseases including brain injury [35, 36]. Meanwhile, some researchers also found that the levels of NF-κB, TNF-α and IL-1β were related with apoptosis of hippocampal neurons [37, 38]. In our study, after the mice were treated by rehabilitation training and TLR4/MyD88 signaling pathway inhibitor, our results indicated that rehabilitation training could effectively reduce the inflammatory level and apoptosis of hippocampal neurons in mice with ischemic stroke by inhibiting TLR4/MyD88 signaling pathway.

At present, animal experiments and clinical studies have confirmed that rehabilitation training can promote the recovery of neurological function in ischemic cerebral damage [39, 40]. In this study, we established a MCAO model and carried out roller training, rotating bar training, and balance beam training on the nerves and muscles of mice to simulate clinical rehabilitation training [41, 42]. At the same time,

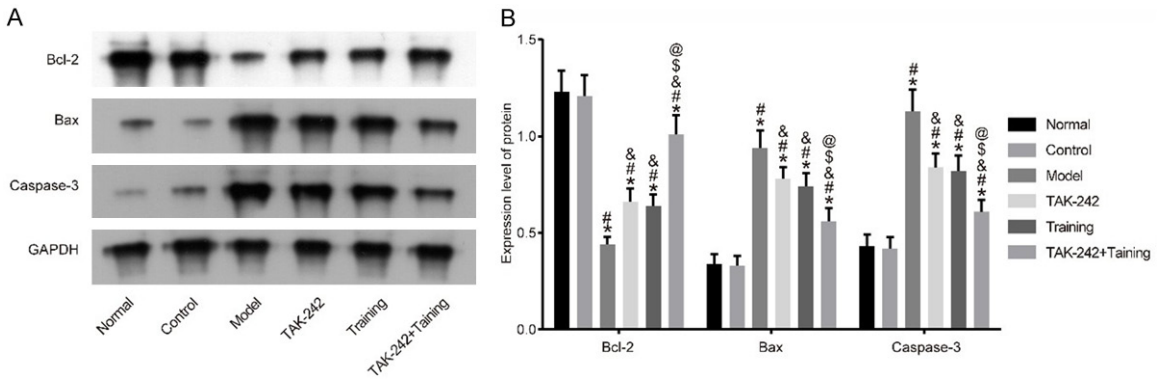

Figure 7. Protein expressions of Bcl-2, Bax, and Caspase-3 detected by Western blot. A: Protein bands; B: Protein expressions. Compared with the normal group, *P<0.05; compared with the control group, #P<0.05; compared with the model group, &P<0.05; compared with the TAK-242 group, $P<0.05; compared with the Training group, @P<0.05.

mice with ischemic stroke were treated with TLR4/MyD88 signaling pathway inhibitor and rehabilitation training. Our results demonstrated that TLR4/MyD88 signaling pathway participates in the anti-apoptotic effect of rehabilitation training on neurons of mice with ischemic stroke by affecting the hippocampal neuronal apoptosis and neurological recovery. This finding may offer some theoretical basis for the clinical treatment of ischemic stroke.

It remains unclear whether the TLR4/MyD88 signaling pathway causes neuronal apoptosis by working in mitochondrial apoptotic pathway, direct regulation of apoptosis-related pathway, or causing cell cycle abnormalities, and further experimental validation is required to determine if TLR4/MyD88 signaling pathway inhibitors can be used in clinical treatment.

Disclosure of conflict of interest

None.

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