European Review for Medical and Pharmacological Sciences 2019; 23: 6321-6329

Simvastatin alleviates inflammation and oxidative stress in rats with cerebral hemorrhage through Nrf2-ARE signaling pathway

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Abstract. – OBJECTIVE: To investigate the regulatory effects of simvastatin on the inflammation and oxidative stress in rats with cerebral hemorrhage through the nuclear factor E2-related factor 2-antioxidant response element (Nrf2-ARE) signaling pathway.

MATERIALS AND METHODS: A total of 120 healthy male rats weighing 280-300 g and 7-8 weeks old were selected to establish the traumatic brain injury (TBI) model. Rats were divided into group A (trauma operation, n=30), group B (no treatment, n=30), group C (drug administration after trauma operation, n=30), and group D (no trauma operation, drug administration, n=30). Cerebral edema content in brain tissues was measured by calculating the dry and wet weight. Neurological dysfunction was scored using the Garcia method. Positive levels of the Toll-like receptor 4 (TLR4) and interleukin-1β (IL-1β) were qualitatively analyzed via immunohistochemistry. Protein levels of TLR4 and IL-1β were quantitatively analyzed via Western blotting. Moreover, the brain injury volume and neuronal apoptosis were evaluated via Nissl staining and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining, respectively. At 48 h after injury, activities of superoxide dismutase (SOD), reduced glutathione (GSH), and oxidized glutathione (GSSG) in brain tissues were detected, and levels of malondialdehyde (MDA) and nitric oxide (NO) were detected using the enzyme activity assay kits. Finally, relative levels of the Nrf2-ARE signaling pathway and its downstream molecules heme oxygenase-1 (HO-1) and NAD (P) H dehydrogenase, quinone 1 (NQO1) were detected via reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Western blotting.

RESULTS: Compared with those in group B, cerebral edema content in brain tissues significantly increased ($p<0.05$), the neurological dysfunction score significantly declined ($p<0.05$), and protein levels of TLR4 and IL-1β were significantly upregulated in group A ($p<0.05$). In group C, relative levels of TLR4 and IL-1β were down-regulated, cerebral edema content decreased, and the neurological dysfunction score significantly increased ($p<0.05$). After 48 h, activities of SOD, reduced GSH and GSSG and levels of MDA and NO all increased, and levels of MDA and NO declined in group C ($p<0.05$). Western blotting and RT-PCR showed that simvastatin could increase the transcriptional level of Nrf2. After simvastatin intervention, expression levels of downstream molecules HO-1 and NQO1 were upregulated.

CONCLUSIONS: Simvastatin alleviates TLR4-mediated inflammatory injury, promotes neurological recovery and resists oxidative stress through the Nrf2-ARE signaling pathway, thus exerting a neuroprotective effect in TBI.

Key Words
Cerebral hemorrhage, Inflammatory response, Oxidative stress, Simvastatin.

Introduction

Cerebral hemorrhage is characterized by proneness to sequelae and difficulty in recovery. Studies have demonstrated that secondary brain injury following cerebral hemorrhage is mainly caused by the inflammatory response and oxidative stress response in brain tissues around the hematoma after onset[1]. Some studies have found that brain trauma induces the inflammatory response in the brain and systemic circulation, and mediates the production of such inflammatory factors as interleukin-1β (IL-1β)[2]. Toll-like receptor 4 (TLR4) and inflammatory factors are activated in tissues of ischemic brain injury[3,4]. TLRs respond to infec-

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tion through recognizing the pathogen-associated molecular pattern, thereby triggering immune response against invading microorganisms[5]. A total of 12 mammalian TLR family members have been identified currently. After activation, nuclear factor E2-related factor 2 (Nrf2) can protect cells from oxidative stress-induced injury and increase cell viability. Once the Nrf2 signaling pathway is destroyed, reduced angiogenesis of endothelial cells and downregulated antioxidant genes lead to oxidative stress response[6].

In the case of tissue damage, produced free radicals interact with some molecules to form active free radicals, which causes damage to the body. The antioxidant defense system in the body resists the damage[7]. The antioxidants are a kind of molecules that can interact with free radicals and terminate the cascade reactions before important molecules are destroyed. Microvascular superoxide free radicals (O_2^-) extensively produced after cerebral hemorrhage[8], and removal of O_2^- can reduce the superoxide levels after trauma and prevent the loss of microvascular self-regulating function. Moreover, the body's antioxidants are activated when oxidative stress response occurs.

In the present study, the effects of simvastatin on the inflammatory response and oxidative stress response in brain tissues after cerebral hemorrhage were explored.

Materials and Methods

Research Materials

Grouping

In this research, 120 healthy male rats (280-300 g in weight, 7-8 weeks old) were purchased from the Laboratory Animal Center of Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats were given free access to food and water. They were randomly divided into group A (trauma operation, n=30), group B (no treatment, n=30), group C (drug administration after trauma operation, n=30), and group D (no trauma operation, drug administration, n=30). This study was approved by the Animal Ethics Committee of Jiamusi University Animal Center.

Experimental materials and reagents

Simvastatin tablets used in the investigation were provided by Zhejiang South Ocean Pharmaceutical Co., Ltd. (Linhai, China); the protein extraction kit from Chuangweishiji (Beijing, China); Nissl and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay kits from Roche (Basel, Switzerland); oxidized glutathione (GSSG), reduced glutathione (GSH) and superoxide dismutase (SOD) activity kits, malondialdehyde (MDA) and nitric oxide (NO) assay kits and rabbit anti-Nrf2 monoclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) kit was provided by Chuangweishiji (Beijing, China), and the primers were synthesized by Sangon (Shanghai, China). The rabbit anti-TLR4 polyclonal antibody was provided by Beijing Bioss Biotechnology Co., Ltd. (Beijing, China); rabbit anti-IL-1β polyclonal antibody from Bioworld Technology Inc. (St. Louis Park, MN, USA); and the immunohistochemistry kit from Fuzhou Maxim Biotechnologies Co., Ltd. (Fuzhou, China).

Methods

Establishment of traumatic brain injury (TBI) model

Rats were anesthetized with 20% procaine injection (5 mL/kg). After 30 min, the brain operation was performed, and the brain injury was caused by the weight impact. Rats in group A were not subjected to weight impact. At 1 h after operation, 1 mL simvastatin injection was intraperitoneally injected in rats of group C and D.

Determination of dry-wet weight of brain tissues

After the rats were executed, 100 mg brain tissues close to hematoma were harvested, and the wet weight was measured. The tissues were baked in an electrothermostat at 100°C, and the weight was measured after about 48 h. The tissues continued to be baked until the weight remained unchanged. Cerebral edema content = dry weight – wet weight/wet weight.

Neurological dysfunction score using the Garcia method

Neurological dysfunction was graded using the double-blind method, and the scoring items and criteria were shown in Table I.

Immunohistochemistry

A total of 12 rats in each group were anesthetized and executed. Brain tissues were taken, fixed, dehydrated, and prepared into sections. The sections were deparaffinized and hydrated at room temperature, followed by heat antigen retrieval, inactivation with 3% hydrogen peroxide, and sealing

Table I. Neurological dysfunction score using the Garcia method.

Item	3 points	2 points	1 point	0 point
Autonomic movement	Normal	Mildly affected	Severely affected	No movement
Tail-suspension four-limb movement	Symmetrical	Non-symmetrical	Hemiplegia	
Forelimb stretching at table edge	Symmetrical	Mildly non-symmetrical	Severely non-symmetrical	Hemiplegia
Climbing and grasping ability	Climb easily	One-side damage	Inability to climb or circle	
Somatosensory response	Bilateral symmetrical	One-side slow response	One-side no response	
Beard-touching response	Symmetrical	Non-symmetrical	One-side no response	

of non-specific sites. The sections were incubated with the rabbit anti-mouse TLR4 and rabbit anti-mouse IL-1β at 4°C overnight. On the next day, the secondary antibody was added for incubation at room temperature for 20 min. 50 μL of streptavidin-peroxidase solution was applied on the sections and incubated at room temperature for 10 min. The staining was terminated with 100 μL of diaminobenzidine (DAB) solution (Solarbio, Beijing, China), and the sections were counterstained with hematoxylin, followed by differentiation, dehydration, airing, and sealing. Finally, the sections were photographed under a microscope.

Nissl and TUNEL staining

The integrity of the brain was identified *via* Nissl staining. The brain tissue sections were soaked in 0.5% gelatin, pasted on the gelatin-treated glass slide, and placed in 0.5% toluidine blue at room temperature for 30 min. Then, the sections were rinsed with distilled water and placed in 75%, 80%, and 95% alcohol for 1 min, respectively, followed by color separation using the special separating solution. Finally, the sections were soaked in 100% alcohol, immersed in xylene, sealed with neutral balsam, and photographed under the microscope.

Positive expression was identified *via* TUNEL staining. The paraffin sections were prepared, deparaffinized, hydrated, and placed in transparentization solution for 8 min. After the glass slide was dried, sections were incubated with 50 μL of TUNEL staining solution and rinsed. Afterwards, sections were incubated with 50 μL of Converter-POD and rinsed again. Then, the sections were counterstained with hematoxylin, rinsed, dehydrated with alcohol in gradient concentration, transparentized with xylene, and sealed with neutral balsam. Finally, a drop of glycerol was added, and the sections were observed under the microscope.

Determination of antioxidant enzyme activity and oxidative stress products

The brain tissues were taken at 48 h after drug administration, and the activities of SOD, reduced GSH and GSSG, as well as the levels of MDA and NO, were detected using the enzyme activity assay kits.

For SOD detection, the brain tissues were centrifuged for preparing the homogenate. SOD content in tissue homogenate was determined by the reduction of the chromophoric group.

For reduced glutathione detection, 0.2 mL of brain tissue homogenate was incubated with 0.2 mL of reduced GSH working solution and mixed evenly, followed by a water bath. 0.1 mL of 37°C oxidizing agent was added and mixed evenly, followed by a water bath. 2 mL of acidic precipitant was added and centrifuged, and 1 mL of homogenate was taken and added with 1.25 mL of reduced GSH detection buffer and 0.25 mL benzoic acid developing solution. Finally, the absorbance was measured at a wavelength of 422 nm using an ultraviolet spectrophotometer.

For GSSG detection, 10 μL of serum to be detected and 200 μL of reduced GSH working solution were added into each well of a 96-well plate, and 50 μL of NADPH working solution (0.12 mg/mL) was also added, followed by incubation at room temperature for 5 min. Then, 10 μL of serum to be detected and 200 μL of T-GSH working solution were added into another 96-well plate, and 50 μL of NADPH working solution (0.12 mg/mL) was also added, followed by incubation at room temperature for 5 min. The absorbance was immediately measured at a wavelength of 410 nm using a microplate reader for recording the reduced GSH and T-GSH concentrations. The GSSG content = T-GSH content – GSH content.

For MDA detection, 40 μL of samples to be detected were added into the enzyme-coated plate, sealed with a membrane, incubated and washed

after 30 min. Then, 50 μL of enzyme-labeled reagent was added into each well, followed by incubation and washing. 50 μL of developing agent A and 50 μL of developing agent B were added into each well for color development in a dark place at 37°C for 15 min. Finally, 50 μL of termination buffer was added into each well, and the absorbance was measured at a wavelength of 450 nm.

For NO detection, 0.1 mL of sample and 0.4 mL of mixed reagent were added and mixed evenly, followed by a water bath at 37°C for 1 h. 0.2 mL of reagent 3 and 0.1 mL of reagent 4 were added, mixed evenly for 30 s, placed at room temperature for 40 min, and centrifuged for 10 min. 0.5 mL of supernatant was taken, added with 0.6 mL developing agent, and placed at room temperature for 10 min. The absorbance was measured at a wavelength of 550 nm.

RT-PCR

The RNA was extracted from the brain tissues, reversely transcribed and heat-inactivated at 95°C, followed by RT-PCR. Three replicates were set for PCR. 8 μL of ultrapure water, 2.5 μL of dNTPs, 0.4 μL of primer, 0.5 μL of RNasin, and 2.5 μg template were added into the reaction system. After reaction at 70°C for 5 min, the mixture was rapidly cooled with ice water and added with 5 μL of reverse transcription buffer and 1 μL of reverse transcriptase, followed by reaction at 37°C for 5 min and at 90°C for 5 min. The primer sequences were shown in Table II.

Detection of protein expression levels via Western blotting

Protein levels of TLR4, IL-1β, and Nrf2 were detected as follows: a total of 12 rats were randomly selected from each group and executed, and the brain tissues were taken from the same site and added with buffer for homogenization and centrifugation. After complete precipitation, the supernatant was taken to detect the protein concentration using the bicinchoninic acid (BCA) method (Abcam, Cambridge, MA, USA). The protein sample was loaded for 12% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) under 120 V for 60 min (10% SDS-PAGE was applied for determining Nrf2 level). The standard substance was used as a control. Then, the protein was transferred onto the cellulose membrane under 120 V for 90 min. The membrane was cut and incubated with the IL-1β antibody (1:400), TLR4 antibody (1:1000), and Nrf2 antibody (1:1000), followed by incubation at 4°C overnight. On the next day, the membrane was rinsed, sealed with 5% skim milk powder, and incubated with the secondary antibody at room temperature for 20 min. Finally, the color was developed using enhanced chemiluminescence (ECL).

GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was used as an internal reference.

Table II. Primer sequences.

mRNA	Sequence
Nrf2	F: 5'-CCCGGCGGCGGCAGCTCCAA-3' R: 5'-AACAGTCATAATAATCCTTT-3'
HO-1	F: 5'-GAACTCCGGCTCCGAGAACT-3' R: 5'-CCAACAAAAACAAGACAGAA-3'
NQO1	F: 5'-AGGGCTCTTCTCGCCGCCAT-3' R: 5'-CTATTTTCTAGCTTTGATCT-3'

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical processing. χ^2-test and *t*-test were performed for the comparison of multiple sample rates in row-column tables. $p<0.05$ suggested that the difference was statistically significant.

Results

Analysis of Cerebral Edema Content in Brain Tissues

The cerebral edema in brain tissues of TBI rats (group A and C) was significantly higher than that in blank group (group B and D) ($p<0.05$). Moreover, the cerebral edema was lower in group C than that in group A ($p<0.05$), and the difference was statistically significant (Figure 1).

Neurological Dysfunction Score Using Garcia Method

Evident neurobehavioral disorders could be seen in group A, and the score was far lower than that in group B and D ($p<0.05$). The neurological dysfunction score in group C significantly increased compared with that in group A, and the difference was statistically significant ($p<0.05$) (Figure 2).

Immunohistochemistry

More TLR4-positive and IL-1β-positive cells were observed in group A and C than that in group B and D ($p<0.05$), but they were fewer in group C than that in group A ($p<0.05$) (Table III, Figure 3).

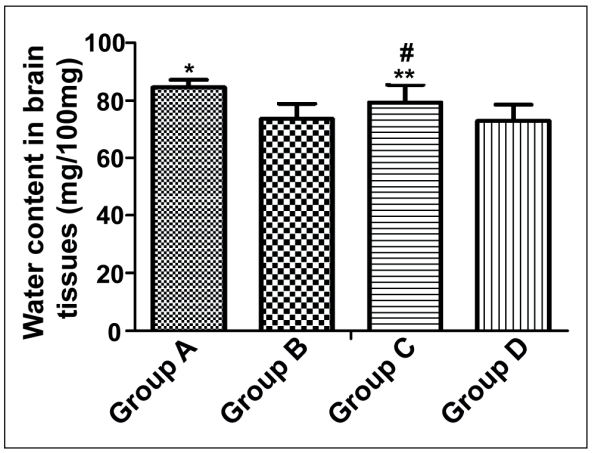

Figure 1. Cerebral edema in the brain tissues of the four groups. ***A***, Trauma operation; ***B***, No treatment; ***C***, Drug administration after trauma operation; ***D***, No trauma operation, drug administration. *The cerebral edema in brain tissues is significantly pronounced than that in group B and D ($p<0.05$). **The cerebral edema in brain tissues is significantly pronounced than that in group B and D ($p<0.05$). #The cerebral edema in brain tissues is much attenuated than that in group A ($p<0.05$).

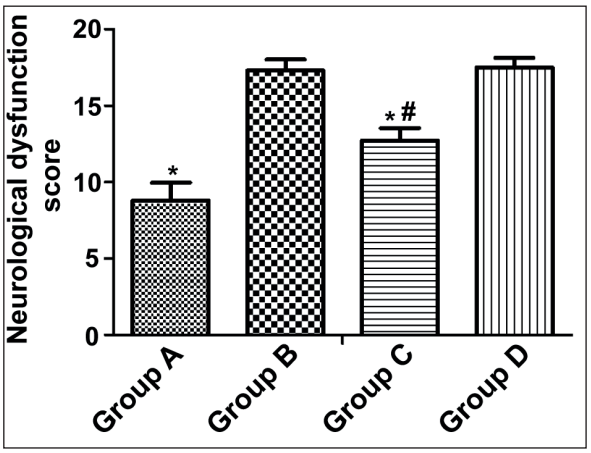

Figure 2. Neurological dysfunction score in the four groups. ***A***, Trauma operation; ***B***, No treatment; ***C***, Drug administration after trauma operation; ***D***, No trauma operation, drug administration. *The neurological dysfunction score is significantly lower than that in group B and D ($p<0.05$). **The neurological dysfunction score is significantly lower than that in group B and D ($p<0.05$). #The neurological dysfunction score is higher than that in group A ($p<0.05$).

Table III. Comapriosn of number of TLR4- and IL-1β-positive cells among the four groups (n=12, $\bar{\chi}\pm s$).

Group	TLR4	IL-1β
A	32.83 ±2.57	30.53 ±2.76
B	5.33 ±0.42[a]	6.45 ±0.48[a]
C	17.45±1.88[b]	18.09±1.58[b]
D	5.65±0.39[a]	5.70±0.44[a]

[a]$p<0.05$, [b]$p<0.05$

Nissl and TUNEL Staining

At 48 h after trauma, the area and proportion of Nissl- and TUNEL-stained regions were elevated in group A ($p<0.05$), which were smaller in group C than those in group A ($p<0.05$) (Table IV).

Antioxidant Enzyme Activities and Oxidative Stress Product Levels

At 48 h after trauma, the levels of antioxidant enzymes and oxidative stress products in group A were markedly higher than those in group B and

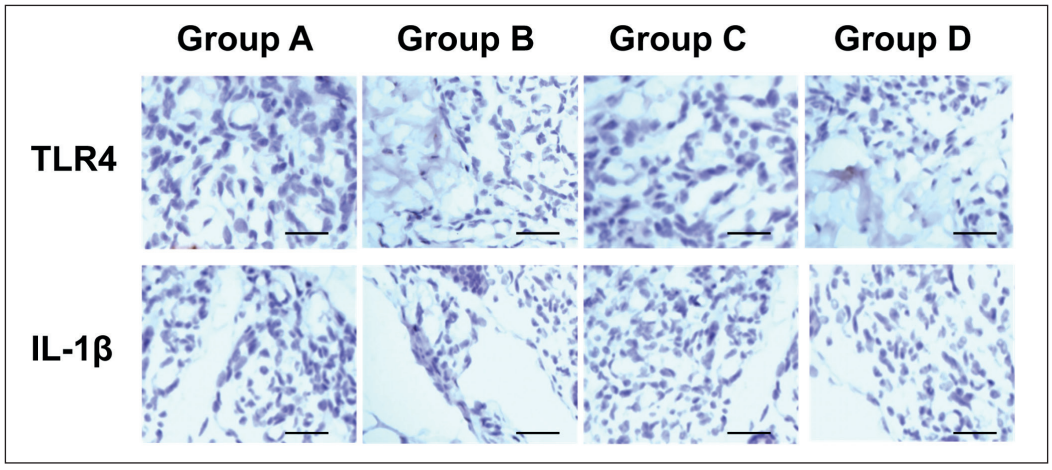

Figure 3. Immunohistochemical staining of TLR4 (upper row) and IL-1β (bottom row) (magnification: 100×).

Table IV. Neuronal degeneration (n=6, $\bar{\chi}$±s).

Group	Damage area (*10^2 mm^2/ 400× field)	Number of apoptotic cells (*102/400× field)
A	31±2.6	8.7±0.8
B	15±1.4[a]	3.2±0.4[a]
C	20±1.8[b]	5.1±0.7[b]
D	13±1.1[a]	3.0±0.4[a]

[a]p<0.05, [b]p<0.05

D (p<0.05). After simvastatin intervention, the activities of antioxidant enzymes SOD, reduced GSH and GSSG increased (p<0.05), while the levels of oxidative stress products MAD and NO significantly declined (p<0.05) (Table V).

Nrf2-ARE Signaling Pathway Detected Via RT-PCR

Relative levels of Nrf2-ARE and downstream genes in group A were higher than those in group B and D. Their levels were further upregulated after simvastatin intervention (Figure 4).

Protein Levels Detected Via Western Blotting

Protein levels of TLR4, IL-1β, and Nrf2 in each group were determined. As shown in Figure 5, protein levels of TLR4 and IL-1β were higher in group A and group C than those in group B and D, which were higher in group C than those in group A. The results of Western blotting showed that simvastatin did not alter the expression of Nrf2.

Discussion

The present study revealed that simvastatin administration could enhance the anti-inflammatory and oxidative stress responses of brain tissues, reduce the edema and apoptosis of brain tissues, improve the neurological function and activity of TBI rats. The above results showed that simvastatin can improve the symptoms of a cerebral hemorrhage. Statins block the mevalonate pathway and reduce the plasma cholesterol, which is often applied clinically to lower blood lipids. Subsequent studies have found that mevalonate is also able to regulate cellular signal transduction and activation of transcription factors. Moreover, studies have demonstrated that statins can effectively reduce neurological deficits in the rat model of middle cerebral artery occlusion[9]. Therefore, simvastatin provides new ideas for the treatment of cerebral hemorrhage and neurological dysfunction.

Cerebral hemorrhage can lead to brain edema, apoptosis and inflammatory response of brain tissues, causing serious sequelae. Studies have shown that the inflammatory response occurs after a cerebral hemorrhage, which is one of the important factors of secondary brain injury[10]. The massive expressions of inflammatory factors, including IL-1, IL-6, and TNF-α, induce a series of actions in brain tissues, leading to neurological dysfunction after cerebral hemorrhage[11,12]. At the same time, researches have found that TLR4 is activated after TBI, thereby inducing the massive expressions of inflammatory factors such as IL-6, TNF-α, and IL-1β[13]. In this study, expression levels of TLR4 and the inflammatory factor IL-1β were upregulated in brain tissues of TBI rats, which were further elevated after simvastatin intervention, proving that simvastatin exerted an anti-inflammatory effect through promoting TLR4. Also, cerebral edema content in brain tissues and apoptosis significantly declined in TBI rats after simvastatin intervention, further indicating that simvastatin could reduce the nerve damage caused by cerebral hemorrhage.

In the damaged nervous system, many potential O_2^- sources may only work within the first few minutes and hours after injury, including the auto-oxidation of arachidonic acid cascade enzyme and biogenic amine neurotransmitters, "mitochondrial leakage", activation of xanthine oxidase, and oxidation of exosmic hemoglobin. After injury of activated microglia, infiltrating

Table V. Antioxidant enzyme activity and oxidative stress product expressions (n=6, $\bar{\chi}$±s).

Group	SOD	GSH	GSSG	MAD	NO
A	1.5±0.07	1.3±0.12	1.5±0.09	4.4±0.28	3.7±0.33
B	1.0±0.13[a]	1.0±0.33[a]	1.0±0.22[a]	1.0±0.12[a]	1.0±0.08[a]
C	3.3±0.24[b]	2.3±0.18[b]	2.7±0.25[b]	1.8±0.19[b]	1.5±0.13[b]
D	1.2±0.14[a]	0.98±0.07[a]	1.1±0.08[a]	1.2±0.07[a]	1.3±0.16[a]

[a]p<0.05, [b]p<0.05

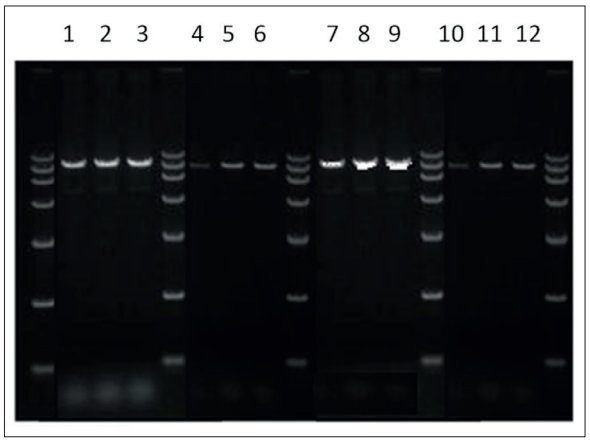

Figure 4. No. 1-3 bands: mRNA expressions of Nrf2, HO-1, and NQO1 in group ***A***, No. 4-6 bands: group ***B***, No. 7-9 bands: group ***C***, and No. 10-12 bands: group D.

neutrophils and macrophages, a large amount of O_2^- is produced, and spontaneously decomposed into H_2O_2 under the catalysis of SOD. The antioxidant enzymes in cells also include glutathione peroxidase (GPx) and glutathione reductase (GR). During the process in which GPx converts peroxides into the non-toxic form, reduced GSH is oxidized to be GSSG, and then, GR and GSSG contribute to the production of reduced GSH. GSH-S transferase can detoxify harmful substances, and glucose-6-phosphate dehydrogenase provides electron donors for the antioxidant defense system. Enzymes that remove superoxide and H_2O_2 can protect cells from oxidative stress, but the system could be damaged when the production of O_2^- and H_2O_2 exceeds the normal threshold. The above process is the main mechanism of superoxide free radical scavenging[14]. The synaptic function declines due to the oxidative stress and dysfunctional transport of mitochondria to the synaptic region[15], leading to neurodegeneration after brain injury[16]. MDA is one of the commonly-used indexes for determining lipid peroxidation[17]. During the oxidative stress, MDA is produced and Ca^{2+} level increases due to membrane lipid peroxidation, leading to vascular endothelial

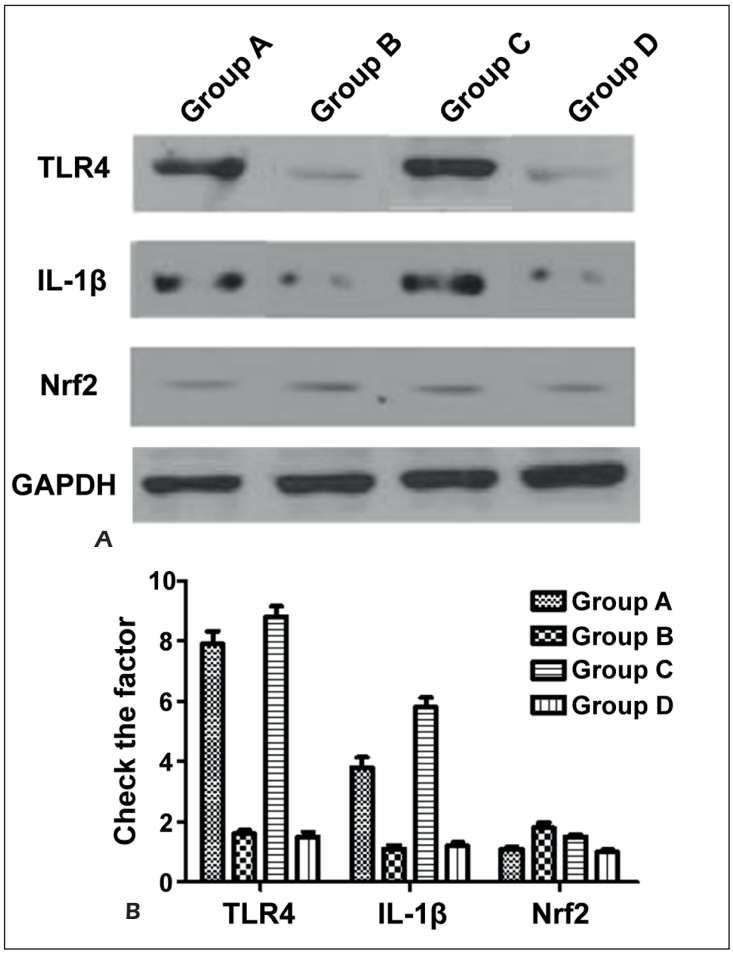

Figure 5. ***A***, Western blotting of brain tissues in the four groups. ***B***, Protein levels of TLR4 and IL-1β in TBI rats are significantly higher than those in blank group ($p<0.05$). Protein levels of TLR4 and IL-1β further increase after simvastatin treatment ($p<0.05$), while Nrf2 has no significant difference ($p>0.05$).

injury, reducing NO secretion and damaging vasoconstriction function[18]. In this work, therefore, the oxidative stress response of tissues was identified by determining levels of SOD, reduced GSH and GSSG.

Transcription levels of Nrf2 and its downstream molecules HO-1 and NQO1 were up-regulated in brain tissues of TBI rats, and they were further elevated after simvastatin administration. It is suggested that simvastatin can affect the Nrf2-ARE pathway, increase the expression of its downstream signaling molecules and resist the oxidative stress. Besides, simvastatin can increase levels of antioxidant enzymes SOD, reduced GSH and GSSG. However, the stimulated effect lasts for a short period, and eventually fails to against the oxidative stress injury[19,20]. Therefore, expression levels of antioxidant enzymes need to be lastingly up-regulated by drugs. MDA and NO are oxidative stress products that cause damage to nerves. In TBI rats receiving simvastatin intervention, the expression levels of antioxidant enzymes increased, while the oxidative stress products MDA and NO declined. Therefore, it can be determined that simvastatin resisted the oxidative stress through up-regulating the expression of antioxidant enzymes, thereby exerting a neuroprotective effect.

Transcriptional level of Nrf2 was elevated by simvastatin intervention, while its protein level did not change. It is believed that simvastatin could only stimulate the Nrf2-ARE pathway at the nucleic acid level. Simvastatin may become a drug in the treatment of cerebral hemorrhage and improvement of neurological function, which provides a new therapeutic method for patients with cerebral hemorrhage.

Conclusions

We demonstrated that simvastatin alleviates TLR4-mediated inflammatory injury, promotes neurological recovery and resists oxidative stress through the Nrf2-ARE signaling pathway, thus exerting a neuroprotective effect in TBI.

Funding Acknowledgements

This study was supported by the project 'Plasmid pVAX1-Mediated Adrenomedullin Gene Therapy for Cerebral Vasospasm Following Subarachnoid Hemorrhage in Rats' (2016-KYYWF-0579).

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Lei C, Lin S, Zhang C, Tao W, Dong W, Hao Z, Liu M, Wu B. High-mobility group box1 protein promotes neuroinflammation after intracerebral hemorrhage in rats. Neuroscience 2013; 228: 190-199.
2) Venetsanou K, Vlachos K, Moles A, Fragakis G, Fildissis G, Baltopoulos G. Hypolipoproteinemia and hyperinflammatory cytokines in serum of severe and moderate traumatic brain injury (TBI) patients. Eur Cytokine Netw 2007; 18: 206-209.
3) Chen L, Yu CX, Song B, Cai W, Liu C, Guan QB. Free fatty acids mediates human umbilical vein endothelial cells inflammation through toll-like receptor-4. Eur Rev Med Pharmacol Sci 2018; 22: 2421-2431.
4) Hamanaka J, Hara H. Involvement of Toll-like receptors in ischemia-induced neuronal damage. Cent Nerv Syst Agents Med Chem 2011; 11: 107-113.
5) Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. Biochem Biophys Res Commun 2009; 388: 621-625.
6) Chen X, Yan L, Guo Z, Chen Z, Chen Y, Li M, Huang C, Zhang X, Chen L. Adipose-derived mesenchymal stem cells promote the survival of fat grafts via crosstalk between the Nrf2 and TLR4 pathways. Cell Death Dis 2016; 7: e2369.
7) Huang J, Shen XD, Yue S, Zhu J, Gao F, Zhai Y, Busuttil RW, Ke B, Kupiec-Weglinski JW. Adoptive transfer of heme oxygenase-1 (HO-1)-modified macrophages rescues the nuclear factor erythroid 2-related factor (Nrf2) antiinflammatory phenotype in liver ischemia/reperfusion injury. Mol Med 2014; 20: 448-455.
8) Kontos HA, Wei EP. Superoxide production in experimental brain injury. J Neurosurg 1986; 64: 803-807.
9) Chen J, Zhang ZG, Li Y, Wang Y, Wang L, Jiang H, Zhang C, Lu M, Katakowski M, Feldkamp CS, Chopp M. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Ann Neurol 2003; 53: 743-751.
10) Kang K, Kim YJ, Kim YH, Roh JN, Nam JM, Kim PY, Ryu WS, Lee SH, Yoon BW. Lithium pretreatment reduces brain injury after intracerebral hemorrhage in rats. Neurol Res 2012; 34: 447-454.
11) Liew HK, Pang CY, Hsu CW, Wang MJ, Li TY, Peng HF, Kuo JS, Wang JY. Systemic administration of urocortin after intracerebral hemorrhage reduces neurological deficits and neuroinflammation in rats. J Neuroinflammation 2012; 9: 13.
12) Miao X, Liu X, Yue Q, Qiu N, Huang W, Wang J, Xu Y, Zhang Y, Yang J, Chen X. [Deferoxamine suppresses microglia activation and protects against secondary neural injury after intracerebral hemorrhage in rats]. Nan Fang Yi Ke Da Xue Xue Bao 2012; 32: 970-975.

13) Chen G, Shi J, Jin W, Wang L, Xie W, Sun J, Hang C. Progesterone administration modulates TLRs/NF-kappaB signaling pathway in rat brain after cortical contusion. Ann Clin Lab Sci 2008; 38: 65-74.
14) Okatani Y, Wakatsuki A, Kaneda C. Melatonin increases activities of glutathione peroxidase and superoxide dismutase in fetal rat brain. J Pineal Res 2000; 28: 89-96.
15) Povlishock JT, Kontos HA. The role of oxygen radicals in the pathobiology of traumatic brain injury. Hum Cell 1992; 5: 345-353.
16) Kushner D. Mild traumatic brain injury: toward understanding manifestations and treatment. Arch Intern Med 1998; 158: 1617-1624.
17) Liu H, Zhang X, Du Y, Ji H, Li S, Li L, Xing Y, Zhang X, Dong L, Wang C, Zhao K, Ji Y, Cao X. Leonurine protects brain injury by increased activities of UCP4, SOD, CAT and Bcl-2, decreased levels of MDA and Bax, and ameliorated ultrastructure of mitochondria in experimental stroke. Brain Res 2012; 1474: 73-81.
18) Leonarduzzi G, Sottero B, Poli G. Targeting tissue oxidative damage by means of cell signaling modulators: the antioxidant concept revisited. Pharmacol Ther 2010; 128: 336-374.
19) van Muiswinkel FL, Kuiperij HB. The Nrf2-ARE signalling pathway: promising drug target to combat oxidative stress in neurodegenerative disorders. Curr Drug Targets CNS Neurol Disord 2005; 4: 267-281.
20) Vera S, Martinez R, Gormaz JG, Gajardo A, Galleguillos F, Rodrigo R. Novel relationships between oxidative stress and angiogenesis-related factors in sepsis: new biomarkers and therapies. Ann Med 2015; 47: 289-300.