Mechanism of stellate ganglion block anesthesia inhibiting neuronal apoptosis in rats by down-regulating Bax gene

Qiao Xu (qiaox0609@163.com)
The Affiliated People's Hospital of Ningbo University
Lei Li
The Affiliated People's Hospital of Ningbo University
Ke Yan
The Affiliated People's Hospital of Ningbo University
Siqi Ye
The Affiliated People's Hospital of Ningbo University

Research Article

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Abstract

Objective: To explore the feasibility of stellate ganglion block (SGB) for the treatment of brain injury and the inhibitory effect of neuronal apoptosis.

Methods: SD rats were randomly divided into control group, model group (LPS 0.6 mg/kg), and SGB group (LPS 0.6 mg/kg + lidocaine 0.2 mL). The rats were sacrificed after SGB block for 2h to separate the hippocampus. The pathological changes of hippocampal tissue were observed by HE staining, the apoptosis of hippocampal neurons was observed by TUNEL staining, the positive expression of B-cell lymphoma-2-Associated X (Bax) in hippocampal tissue was detected by immunohistochemistry, and the expression of Bax mRNA in hippocampal tissue was detected by qRT-PCR method.

Results: Compared with the control group, the hippocampal tissue of the model group was severely damaged, and the apoptosis rate of hippocampal neurons, the positive rate of Bax protein, the expression of Bax mRNA, and the protein expressions of Baxwere significantly increased, the protein expression of Bcl-2 was significantly decreased (P<0.05).Compared with the model group, the hippocampal tissue damage of the SGB group was significantly reduced, and the hippocampal neuron apoptosis rate, Bax protein positive rate, Bax mRNA expression and Bax protein expressions were significantly decreased, and the expression of Bcl-2 protein was significantly increased (P<0.05).

Conclusion: SGB can reduce the apoptosis of neurons in rats and improve brain injury by inhibiting the expression of Bax gene.

Introduction

Stellate Ganglion block (SGB) refers to the injection of local anesthetic drugs into connective tissue containing Stellate ganglion to reversibly block the excitatory transmission of preganglion and postganglion fibers, thereby maintaining the stability of internal environment and correcting neurological disorders [1–2]. Clinically, it is often used for the treatment of various pains, brain injury, depression, post-traumatic stress disorder and other functional disorders [3–4]. SGB is a minimally invasive form of sympathetic nerve block, which has significant protective effect on various organs [5]. The mechanism of brain injury is complex, involving primary injury and secondary injury. Primary injury is irreversible and has high rate of disability and mortality [6]. Secondary injury often occurs several hours or even days after brain injury, which induces brain edema, neuroinflammation oxidative stress and other pathological changes, resulting in unfavourable prognosis [7]. Pathological reactions caused by brain injury will eventually lead to neuronal degeneration, necrosis and apoptosis [8]. Studies have found that the main cause of cell apoptosis is the overexpression of B-cell lymphoma-2-associated X (Bax) gene in cells [9]. Excessive apoptosis of neurons can easily cause cognitive and behavioral abnormalities in patients, aggravate brain injury and increase disability rate [10]. Therefore, reducing neuronal apoptosis has become the focus of brain injury treatment and improving the prognosis. In this study, the brain injury

model in rats was established by Lipopolysaccharide (LPS), and SGB was used in the model to explore the feasibility and mechanism of SGB in the treatment of neurons apoptosis in brain injury.

1. The Material

1.1 Experimental animals

Thirty SPF male SD rats, with a body weight of (200±20) g, were purchased from Shandong Bo 'an Biotechnology Co., LTD., and the experimental animal license number was SCXK (Lu) 2020-0006. The rats were fed for a week before experiments under the following conditions: temperature 22~25℃, relative humidity 45~60%, day and night duration 12 h:12 h, free drinking water and food. All animal experiments are conducted in accordance with the Laboratory animal management regulations and the “3R principle”.

1.2 Experimental drugs and reagents

LPS (PC1303) was purchased from Beijing Pufei Biotechnology Co., LTD. Lycardoin (A2300) was purchased from Beijing Konarui Biotechnology Co., LTD. HE staining kit (YT8681) was purchased from Beijing Ita Biotechnology Co., LTD. TUNEL Cell apoptosis Detection Kit (FY600017) was purchased from Shanghai Fuyuan Biotechnology Co., LTD. Rabbit anti-rat Bax polyclonal antibody (AB32503), Bcl-2 antibody (AB196495) and enzymole-conjugated sheep anti-rabbit IgG (AB150077) were all purchased from Abcam Company in the UK. DAB Color kit (SW1020) was purchased from Beijing Solebo Technology Co., LTD. ECL luminescent liquid (My35736) was purchased from Beijing Kaishiyuan Biotechnology Co., LTD.

1.3 Experimental Instruments

Leica Biological microscope DM1000 was purchased from Germany Leica Company. Ma-6000 96-well real-time fluorescence quantitative PCR amplification instrument was purchased from Suzhou Yarui Biotechnology Co., LTD. Corning®Axygen® Gel imaging system was purchased from Corning Corporation.

2. Method

2.1 Grouping and treatment of animals

The rats were randomly divided into three groups: control group, model group and SGB group, with 10 rats in each group. Model group and SGB group were intraperitoneally injected with 0.6 mg/kg LPS[11] to induce acute brain injury in rats. Six hours after injection, rats in SGB group were anesthetized by intraperitoneally injected with pentobarbital sodium, fixed on the operating table, and punctured at the spines of the 7th cervical spine of rats. After no blood and no cerebrospinal fluid were extracted, 0.2 mL of 1% lidocaine was injected [12]. The SGB model was successfully established in rats with obvious

Horner syndrome, i.e., ptosis of upper eyelid and wide eye fissure [13], while the control group was only injected with equal volume of normal saline.

2.2 Morris Water Maze experiment [14]

A water maze was prepared and platform quadrant was set. After surgery, the experimental rats were placed in the water maze for adaptive free swimming for 5 min. The rats were trained to find a platform at a fixed point every day for 4 days. On day 5, the time needed to find the platform in each group was recorded as the escape incubation period. If the rats failed to find the platform within 90 s, the escape incubation period was recorded as 90 s, that is, the learning ability of rats in each group was tested. The platform was removed for escape latency experiment from the 7th day after surgery, and the times of crossing the original platform quadrant of rats in each group were recorded to test the memory ability of rats.

2.3 Sample Collection

2 h after SGB block, rats in each group were decapitated and their brains were taken out. After rinsed with pre-cooled normal saline, hippocampal tissue was quickly separated on ice, part of which was fixed in 4% paraformaldehyde for tissue section preparation, and part of which was stored in a refrigerator at -80℃ for later use.

2.4 Histopathological observation of hippocampus

The hippocampal tissues fixed in 4% paraformaldehyde were routinely dehydrated, transparent, paraffin embedded, sliced, stained with HE and sealed with neutral gum. Pathological morphological changes of hippocampal tissues were observed under a microscope, and 5 fields were randomly selected for photography.

2.5 Neuronal apoptosis in hippocampus detected by TUNEL method

The sections of hippocampus prepared in 2.3 were dewaxed and hydrated, protease K was dropped in, and TUNEL reaction solution was added, DAB was used for color rendering, hematoxylin was used to counterstain, observed under the microscope and photographed. Positive neurons, namely apoptotic neurons, were brown, and normal neurons were blue and purple. The apoptosis rate of neurons (number of positive neurons/total number of neurons ×100%) was calculated.

2.6 Bax protein expression in hippocampus detected by immunohistochemistry

Sections of hippocampal tissue prepared in 2.3 were taken for dehydration and antigen repair, sealed with 5% goat serum, and incubated overnight with Bax antibody (1:1000) at 4℃. Then, enzymed-labeled goat anti-rabbit IgG was dropped in, followed by DAB for color rendering. Pictures were taken under microscope, and Image J Image analysis software was used for semi-quantitative analysis. The positive rate of protein (number of positive neurons/total number of neurons ×100%) was calculated.

2.7 Expression of Bax mRNA in hippocampus detected by QRT-PCR

Sections of hippocampal tissue stored in the refrigerator were taken to extract total RNA by TRIzol method and cDNA was obtained by reverse for testing. The reaction conditions were 90℃60 s, 90℃15 s, 65℃30 s and 72℃30 s, with a total of 40 cycles and 60 s extension at 75℃. Related expression of target genes was calculated by $2^{-\Delta\Delta Ct}$ method. Primers required for PCR were: Bax upstream sequence 5 '-GGTCCCGAAGTAGGAAAGGA-3', downstream sequence 5 '-GTCCCGAAGTAGga-AAGGA-3'; GADPH upstream sequence of was 5 '-AccACAGTCCATGCCATcac-3', downstream sequence was 5 '-TCCAccACCCTGTTGCTGTA-3'.

2.8 Expression of Bax and Bcl-2 proteins in hippocampus detected by Western blot

The hippocampal tissue was taken from the refrigerator at -80℃, and the protein was lysed by tissue lysate, and the supernatant was centrifuged. The protein concentration was determined by BCA method. 30 μg protein was sampled, and SDS-PAGE electrophoresis was performed to isolate the protein. Then the protein was transferred to PVDF membrane by wet transfer method, and sealed with 5% goat serum for 1 h. Bax and Bcl-2 primary antibody (1:1000) were added after washing the membrane, and incubated at 4℃ overnight. Primary antibody (1:1000) was added after washing the membrane, and incubated at 37℃ for 2 h without light. After washing the membrane, ECL was developed, and gray values were scanned and analyzed by gel imaging system. Using GAPDH as internal reference, the relative expression levels of target proteins were calculated.

2.9 Statistical Analysis

Graph Prism 8.0 statistical software was used to analyze and process the experimental data, and the measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way AVOVA was used for comparison between multiple groups, and *LSD-t* test was used for comparison between groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effects of SGB on learning and cognitive ability of rats

Compared with the control group, the escape latency of rats in model group was significantly increased, and the times of crossing the original platform quadrant were significantly decreased ($P < 0.05$); Compared with model group, the escape latency of rats in SGB group was significantly shortened, and the times of crossing the original platform quadrant were significantly increased ($P < 0.05$). The results are shown in Table 1.

Tab. 1 Comparison of escape latency and times of crossing the original platform quadrant of rats in each group ($\bar{x} \pm s$, n=10)

Group	Escape latency (s)	Times of crossing original platform quadrant (times)
Control Group	16.02±1.34	5.77±0.38
Model Group	35.22±3.54[a]	2.06±0.16[a]
SGB Group	24.14±2.12[b]	3.12±0.41[b]

Compared with control group, [a]*P*0.05, compared with model group, [b]*P*0.05.

3.2 Effects of SGB on pathological changes of hippocampal tissue and neuronal apoptosis in rats

HE staining results showed that the hippocampal neurons in the control group were normal in shape, with clear structure and orderly arrangement, and no damage was observed. The hippocampal neurons in the model group were obviously damaged, the cells were swollen and disordered, and the nuclei were dissolved. Compared with the model group, the damage of hippocampal tissue in SGB group was significantly reduced, the arrangement of neurons was gradually orderly, and the vacuolar degeneration was reduced, which was similar to the control group. Compared with the control group, the apoptosis rate of neurons in the model group was significantly increased ($P < 0.05$). Compared with model group, the apoptosis rate of SGB group was significantly decreased ($P < 0.05$). The results are shown in Figure 1 and Table 2.

Tab. 2 Comparison of apoptosis rates of hippocampal neurons in each group ($\bar{x}\pm s$, n=5)

Group	Apoptosis rate (%)
Control Group	7.32±0.61
Model Group	36.84±3.33[a]
SGB Group	15.41±1.47[b]

Compared with control group, [a]*P*0.05, compared with model group, [b]*P*0.05.

3.3 Effect of SGB on the positive expression of Bax protein in hippocampus of rats

Compared with control group, the positive rate of Bax protein in hippocampus of model group was significantly increased ($P < 0.05$); Compared with the model group, the positive rate of Bax protein in the hippocampus of SGB group was significantly decreased ($P < 0.05$), as shown in Figure 2 and Table 3.

Tab.3 Comparison of the positive rate of Bax protein in hippocampus of rats in each group ($\bar{x}\pm s$, n=5)

Group	Protein positive rate (%)
Control Group	15.21±1.07
Model Group	41.36±3.82[a]
SGB Group	23.54±2.12[b]

Compared with control group, [a]*P*<0.05, compared with model group, [b]*P*<0.05.

3.4 Effect of SGB on Bax mRNA expression in hippocampus of rats

Compared with control group, the expression of Bax mRNA in hippocampus of model group was significantly increased (P < 0.05); Compared with model group, the expression of Bax mRNA in SGB group was significantly decreased (P < 0.05). The results are shown in Table 4.

Tab. 4 Comparison of Bax mRNA expression in hippocampus of each group ($\bar{x}$±s, n=5)

Group	Bax mRNA
Control Group	1.02±0.10
Model Group	1.74±0.15[a]
SGB Group	1.25±0.12[b]

Compared with control group, [a]*P*<0.05, compared with model group, [b]*P*<0.05.

3.5 Effects of SGB on the expression of Bax and Bcl-2 proteins in hippocampus of rats

Compared with control group, the expression of Bax protein was significantly increased and the expression of Bcl-2 protein was significantly decreased in model group (P < 0.05); Compared with model group, the expression of Bax protein in SGB group was significantly decreased, and the expression of Bcl-2 protein was significantly increased (P < 0.05). The results are shown in Figure 3 and Table 5.

Tab. 5 Comparison of Bax and Bcl-2 protein expression in hippocampus of rats in each group($\bar{x}$±s, n=5)

Group	Bax	Bcl-2
Control Group	0.37±0.04	0.85±0.08
Model Group	1.02±0.10[a]	0.26±0.02[a]
SGB Group	0.71±0.07[b]	0.53±0.05[b]

Compared with control group, [a]*P*<0.05, compared with model group, [b]*P*<0.05.

4. Discuss

The hippocampus is closely related to learning and cognitive functions and is also the most vulnerable part in brain injury [15]. In this study, LPS-induced brain injury was performed in rats. Morris Water Maze test showed that compared with the control group, the escape latency of the rats with brain injury was significantly prolonged and the times of crossing the platform was significantly reduced, indicating that the hippocampal tissue was damaged and the learning ability and cognitive function of the rats were decreased after brain injury, and the model was successfully established. Recent studies have found that cognitive dysfunction is closely related to neuronal apoptosis in hippocampus [16]. Apoptosis is an autonomously ordered programmed cell death controlled by genes, which can maintain the stability of internal environment [17], but abnormal expression of apoptosis is usually involved in the occurrence and development of diseases.

Bax is a pro-apoptotic gene. On the one hand, it can induce the release of signals from organelles and the activation of cysteine protease, antagonize the anti-apoptotic effect of Bcl-2, and promote cell apoptosis and death, namely the death receptor pathway. On the other hand, it acts on the mitochondrial membrane, reduces the membrane potential, improves the permeability of the mitochondrial membrane, induces mitochondrial swelling and damage, and then induces apoptosis, namely the mitochondrial pathway [18–19]. The results of this study showed that the hippocampal tissue of model group was severely damaged and the neuronal apoptosis rate was significantly increased. The expression of Bax mRNA in hippocampal tissue of rats was detected by QRT-PCR and the positive expression of Bax protein was detected by immunohistochemistry. The expression of Bax mRNA and the positive rate of Bax protein in hippocampal tissue of model group were significantly increased, suggesting that the expression of Bax gene and protein were related to hippocampal tissue injury and neuronal apoptosis in rats. This is consistent with the results obtained by Lin Chen et al. in studying neuronal apoptosis in rats with traumatic brain injury [20]. In addition, Western Blot analysis of Bax and Bcl-2 protein expression showed that Bax protein expression was significantly increased and Bcl-2 protein expression was significantly decreased in the hippocampal tissue of the model group, indicating that increased Bax protein expression and decreased Bcl-2 protein expression were involved in the hippocampal neuron apoptosis.

The over-activation of sympathetic nerve can induce the occurrence and development of a variety of cardiovascular and cerebrovascular diseases [21]. SGB can maintain the stability of internal environment and treat pain through local blocking of sympathetic nerve activation [22]. Studies have found that SGB can reduce neuronal apoptosis and autophagy response in ischemia-reperfusion brain injury [23]. In cardiopulmonary bypass surgery, the expression of Bax and Caspase can be reduced by regulating the mitochondrial pathway of hippocampal neurons, alleviating the neuronal apoptosis induced by surgery and improving the cognitive ability of rats. In this study, the SGB model was established on the basis of LPS-induced brain injury in rats. The results showed that the expression of Bax mRNA and the positive rate of Bax protein in the hippocampal tissue of SGB rats were significantly decreased, the expression of Bax protein was significantly increased, the expression of Bcl-2 protein was significantly decreased, the damage of hippocampal tissue was alleviated, and the apoptosis rate was reduced. These results

suggest that SGB can inhibit Bax expression, up-regulate Bcl-2 expression, alleviate hippocampal tissue damage, reduce hippocampal neuron apoptosis, and improve the cognitive function of rats.

In conclusion, by regulating the expression of Bax gene, SGB can promote the activation of Bcl-2, reduce hippocampal neuronal apoptosis, alleviate brain injury and improve cognitive function. The results provide a new method for the treatment and prognosis of neuronal apoptosis and cognitive dysfunction after brain injury in the future.

Declarations

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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Figures

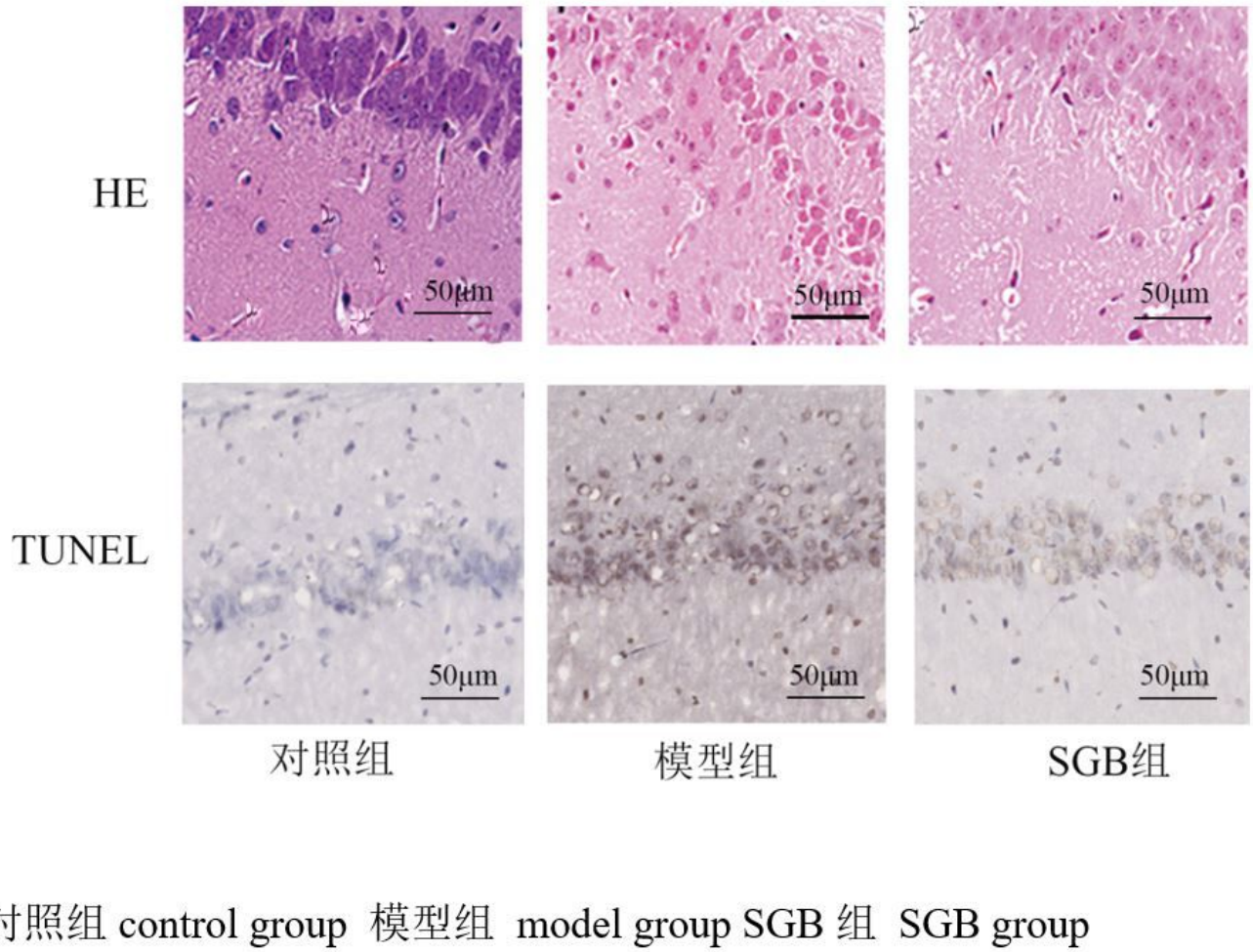

Figure 1

Pathological damage of hippocampal tissue and neuronal apoptosis in each group (×400)

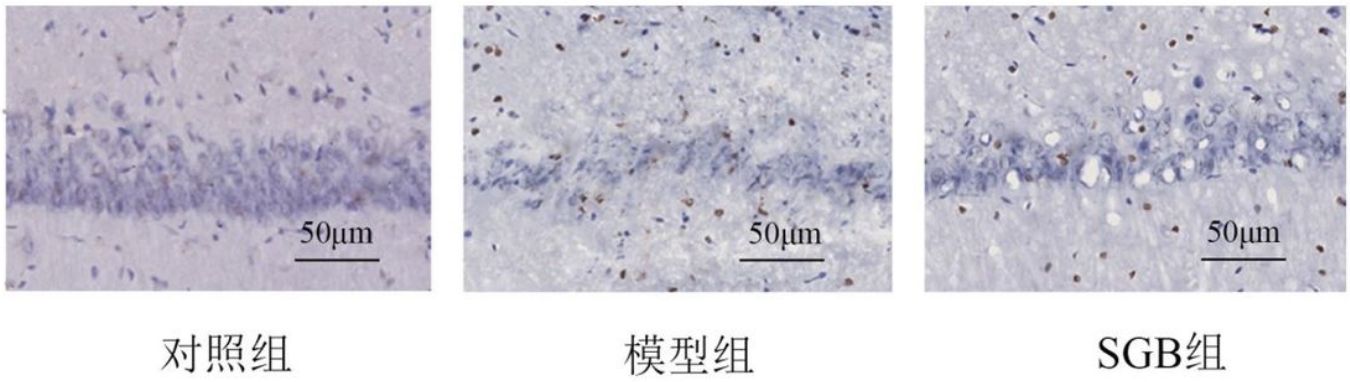

Figure 2

Positive expression of Bax protein in hippocampus of rats in each group (×400)

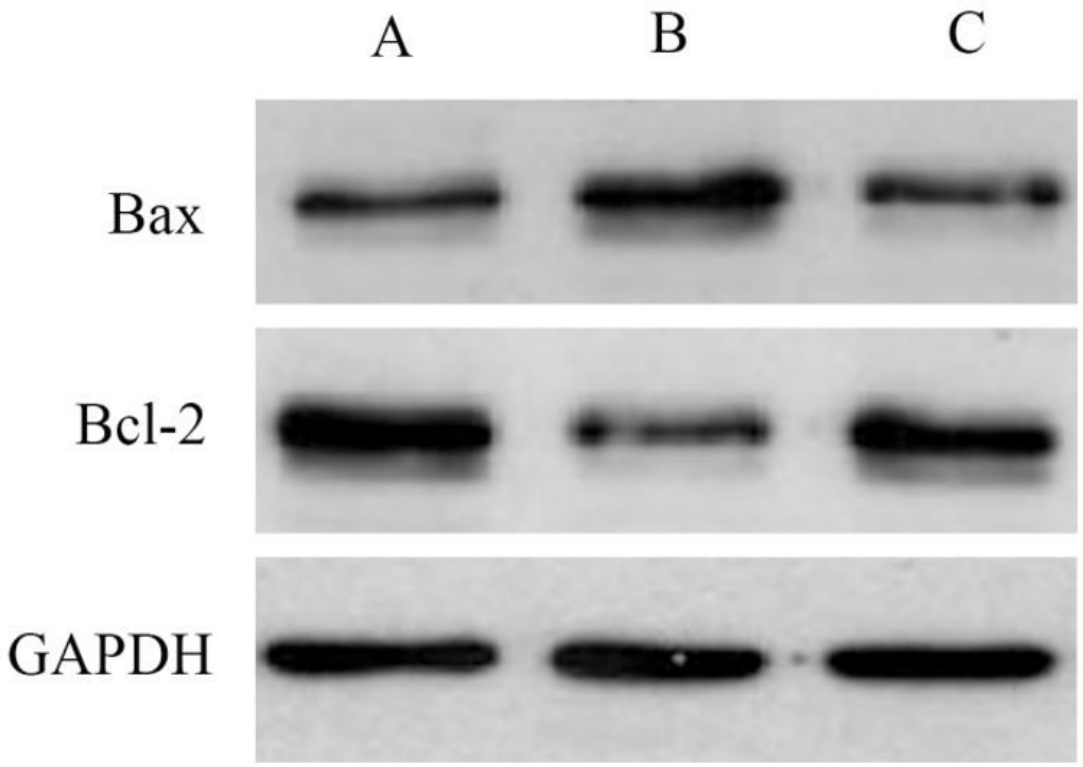

Figure 3

Expression of Bax and Bcl-2 proteins in hippocampus of rats in each group (A is control group; B is model group; C is SGB group)