

Original Article

Neuroprotective effect of hyperbaric oxygen therapy in rats with status epilepticus

Jie Xia¹, Cheng-Yan Li¹, Hui Wang², Qi-Mei Zhang³, Yan-Yun Xiang³

¹Department of Neurology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China; ²Department of Anesthesiology, Renhe Hospital of China Three Gorges University, Yichang 443002, Hubei, China; ³Institute of Neurology, China Three Gorges University, Yichang 443002, Hubei, China

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Abstract: Objective: We investigated the mechanism of neuroprotective effect of hyperbaric oxygen therapy on status epilepticus (SE) in rats. Method: Sixty-three healthy male Sprague-Dawley (SD) rats were randomly divided into two groups: 20 rats in the normal control group, and the remaining 43 rats in SE modeling group by intraperitoneal injection of lithium <http://cn.bing.com/dict/search?q=-&FORM=BDVSP6&mkt=zh-cn> and pilocarpine. After modeling, the rats were further randomly divided into SE group and hyperbaric therapy group. The onset number of spontaneous epilepsy was recorded by behavioral monitoring. The spatial learning and memory ability of rats were tested by Morris water maze. The neuron number in hippocampus region CA1 was measured by HE staining; YKL-40-positive cells were characterized by immunohistochemistry; nerve cell apoptosis was detected by TUNEL assay; mRNA and protein expressions of Bim and YKL-40 were detected using RT-PCR and Western Blotting, respectively. Results: The onset number of spontaneous seizures was greatly reduced in the hyperbaric oxygen therapy group than in the control group and SE group ($P < 0.05$). The spatial learning and memory ability of rats in the hyperbaric oxygen therapy group was obviously improved compared with the control group and SE group according to the test using Morris water maze ($P < 0.05$). Much more neurons were detected in the hippocampus region CA1 in the hyperbaric oxygen therapy group than in the SE group ($P < 0.05$); immunohistochemistry detection showed that YKL-40-positive cells were greatly reduced in the hyperbaric oxygen therapy group ($P < 0.05$); according to real-time PCR and Western Blotting detection, the mRNA and protein expressions of Bim and YKL-40 decreased significantly ($P < 0.05$). Conclusion: Lithium <http://cn.bing.com/dict/search?q=-&FORM=BDVSP6&mkt=zh-cn> and pilocarpine injection can cause the onset of temporal lobe epilepsy. The hyperbaric oxygen therapy may work by blocking the cell apoptosis pathway mediated by Bim and YKL-40 in rats with SE.

Keywords: Hyperbaric oxygen therapy, status epilepticus (SE), rat, neuroprotective effect, mechanism

Introduction

Epilepsy is one of the most common disorders of the nervous system featured by repeated, spontaneous bilateral synchronous discharge due to varying reasons [1, 2]. One prominent result of repeated onset of epilepsy is the loss of neurons and glial cell hyperplasia, causing severe damage to the brain and even persistent neuropsychiatric disorders. Epilepsy can greatly reduce the patient's life quality and may even lead to death. Status epilepticus (SE) refers to the state that the patient does not return to normality between two onsets of seizures. SE may last for over 30 min without stop spontaneously [3-5]. If the seizure cannot be stopped, irreversible damage will be caused to

the brain, especially the hippocampus [6, 7]. Hyperbaric oxygen therapy supplies oxygen to the brain cells, maintaining energy metabolism and preventing the occurrence and progression of nerve cell damage. Many clinical reports have been known over the positive effect of this therapy on traumatic epilepsy [8, 9]. To confirm this, we carried out an animal experiment on the neuroprotective effect of hyperbaric oxygen therapy in rats with SE.

Materials and methods

Experimental animals

Sixty-three healthy male SD rats aged 1 month which had the body weight of 210-220 g were

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Table 1. Reagents, equipments and their sources

Reagents and equipments	Source
Fetal bovine serum	Hangzhou Sijiqing Bioengineering Material Co., Ltd.
Trypsin	Sigma, USA
DMEM	Hyclone, USA
RT-PCR kit, Western Blot kit	Coulter, USA
M-MLV reverse transcriptase	Promega, USA
10% chloral hydrate	Abcam, USA
Bim, CHI3L1 encoding YKL-40 gene, and internal reference GAPDH	Beijing ComWin Biotech Co., Ltd.
Rabbit anti-rat polyclonal antibody against Bim,	Abbiotec, USA
Goat anti-rat biotinylated secondary antibody IgG	BIORAD, USA
DAB color development kit	Wuhan Boster Biological Technology Co., Ltd,
Cell culture plate	Sigma, Germany
Inverted fluorescence microscope	Merck, Germany
Microtome	Leica Instruments Ltd. (Shanghai)
CO ₂ incubator	Changsha Changjin Technology Co., Ltd.
Ultra-clean workbench	Suzhou Antai Air Tech Co., Ltd.

Table 2. Comparison of number of onset of spontaneous seizures between the groups

	Control group	SE group	Hyperbaric oxygen therapy group (n=20)
Duration of seizures (h)	0.00±0.00	20.46±0.23	1.23±0.34

Note: P>0.05 compared with the control; P<0.05 compared with the SE group.

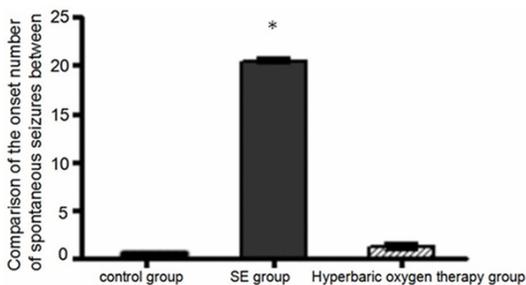


Figure 1. Comparison of the onset number of spontaneous seizures among the groups. *Compared to control group and hyperbaric oxygen therapy group, P<0.05.

selected. They were reared under room temperature and humidity of about 50%. The rats were allowed free access to food and water (ordinary rat feed and clean running water). The SD rats were purchased from Animal Experimental Center of Wuhan University School of Medicine [license No: SCXK [Hubei] 2003-0003]. The procedures of the animal experiment conformed to animal ethics.

Reagents and equipments (**Table 1**).

Method

SE modeling and hyperbaric oxygen therapy: SE was induced by injection of lithium <http://cn.bing.com/dict/search?q=-&FO-RM=BDVSP6&mkt=zh-cn> and pilocarpine (L1-PILO). The abdomen skin was disinfected using

iodophor-soaked cotton ball in rats immobilized on the ultra-clean workbench. Then intraperitoneal injection of 1 mg/kg atropine sulfate was performed to counteract the peripheral cholinergic reaction. This was followed by 30 mg/kg L1-PIL injection 30 min later. The reactions of the rats were observed. If no onset above IV class occurred 30 min later, additional dose of 10 mg/kg was injected every 15 min until persistent SE above IV class was observed. One hour after the onset of SE, 3.5% chloral hydrate was intraperitoneally injected at 1 ml/kg to the rats to stop the seizure and reduce the death. The seizure was classified according to the Racine score: class 0, normal behavior, without spasm and jerk; class I, spasm and twitching of facial muscles responsible for chewing and raising the beard, with solitary myoclonic jerks; class II, twitching of neck muscles, typically nodding; class III, clonus and twitching of unilateral forelimb; class IV, generalized tonic-clonic seizures and myoclonic seizures with body erecting or falling down; class V, repeated onset of the seizures of class IV, presenting as SE or death from seizures. SE modeling was considered successful if SE of class IV was induced.

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Grouping: Among 63 healthy male SD rats, 20 rats were randomly selected as normal controls; the remaining 43 SD rats received intraperitoneal injection of L1-PILO to induce SE. Then the rats with SE were further divided into SE group and hyperbaric oxygen therapy group randomly. The control group was treated by intraperitoneal injection of normal saline, and the onset of seizures was observed. For the SE group, intraperitoneal injection of 1 mg/kg atropine sulfate was performed. After 30 min, 30 mg/kg PILO was injected and the reaction of the rats was observed.

Hyperbaric oxygen therapy: The SE rats were placed into the hyperbaric oxygen cabin 4 h after modeling. The cabin was first washed with pure oxygen for 15 min and then pressurized to 0.2 Mpa at the constant rate of 0.01 Mpa/min. The pressure was maintained for 30 min. Pure oxygen was injected into the cabin, and the oxygen concentration maintained above 96.5%. Then the pressure was reduced at a constant rate to normal pressure 10 min later. After the therapy, the rats were reared conventionally. The therapy was administered four times a day for 3 d.

Monitoring of the onset number of spontaneous seizures

The onset number of spontaneous seizures was recorded within 30 d after modeling using electronic monitoring. Since the experimental animals were sensitive to odor, air exchange was performed 8-10 times per hour in the experimental chamber. Constant illumination was maintained in the chamber and the noise level was below 60 dB; the ambient temperature was 19-26°C, and the relative humidity was 40%-70% in the chamber.

Measurement of spatial learning and memory ability of rats using Morris water maze

Morris water maze was composed of a circular pool, a safety island and recording system. The experiment tested the navigation and exploration ability, and water temperature maintained at about 22°C. The test was performed 23-28 d after modeling. Navigation ability test lasted for 5 d and a half. The time spent by the rat from entering the water to finding and crawling over the platform (safety island) was recorded, i.e., the length of escaping latency(s). Exploration

ability test was performed on the last day (5th day) with the removal of the platform. The rats swam freely in the water for 2 min, and the number of times of passing the platform was recorded within 120 s.

Immunohistochemistry staining for YKL-40

From each group 5 rats were randomly selected 2 w after modeling. The hippocampus tissues were harvested and fixed in 40 g/L paraformaldehyde for 10 min. The tissues were made into frozen sections conventionally and dewaxed with xylene. Next the sections were hydrated in gradient of anhydrous ethanol, and washed with distilled water and subsequently with PBS for 3 min. Then 3% hydrogen peroxide was added to incubate the cells at room temperature for 10 min so as to eliminate endogenous peroxidase activity. Heat-induced antigen repair was performed for 20 min. After cooling to room temperature, the cells were washed with PBS 3 times for 5 min each time. Immunohistochemistry staining was performed according to the manual of BrdU kit. The sections were incubated with rabbit anti-rat monoclonal antibody against YKL-40 (diluted in TBST, 1:1000) and rabbit anti-rat polyclonal antibody against GAPDH (diluted in TBST, 1:1000) at 4°C overnight in a wet box. Then the sections were washed with PBS three times for 5 min each time. Incubation was carried out by adding the corresponding goat anti-rat biotinylated secondary antibody IgG (1:1000) at room temperature for 1 h. After that, PBS was discarded and 100 µl newly prepared DAB reagent was added into each slide. The positive nuclei were stained brownish yellow 10 min later under the microscope. The sections were washed thoroughly with distilled water three times for 2 min each time. After dehydration through gradient alcohol and transparentization in xylene, the sections were left to air dry. The slides were sealed with neutral resin. For each slide, 5 visual fields were randomly selected under the high-power microscope (×200). The BrdU-positive cells were counted in each visual field using the fluorescence microscope, and the average cell count was calculated as the final result.

Measurement of the number of neurons in hippocampus region CA1

The hippocampus tissues were fixed in 40 g/L paraformaldehyde and made into frozen sec-

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Table 3. Comparison of escaping latency in the navigation ability test between the groups (n=20, $\bar{x} \pm s$)

Group	1 d	2 d	3 d	4 d	5 d
Control group	25.21±1.73	20.15±1.38	18.36±1.42	10.23±1.15	7.94±1.21
SE group	52.16±1.68*	35.07±1.46*	30.24±1.50*	16.07±1.18*	12.45±1.27*
Hyperbaric oxygen therapy group	27.16±1.45*, ^a	20.01±1.40*, ^a	10.06±1.46*, ^a	8.05±1.23*, ^a	6.92±1.24*, ^a

Note: *P<0.05 compared with the control; *,^aP<0.01 compared with the control; ^aP<0.05 compared with SE group.

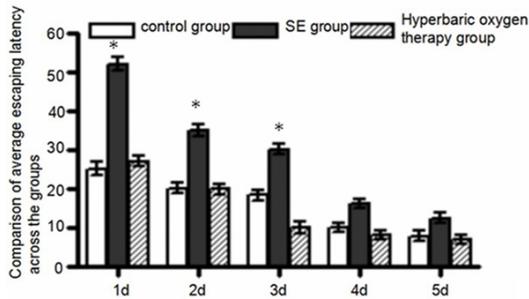


Figure 2. Comparison of average escaping latency across the groups. *Compared to control group and hyperbaric oxygen therapy group, P<0.05.

tions conventionally. After dewaxing with xylene, the sections were dehydrated through alcohol gradient (70%, 80%, 95%, anhydrous ethanol). The sections were washed with distilled for 20 min and counterstained with hematoxylin. After stained with alcoholic solution of eosin, the sections were transparent with xylene. The slides were sealed with neutral gum, dried in the thermostatic oven and observed under the microscope, and pictures were taken. For each slide, 5 non-overlapping visual fields were selected in the hippocampus region CA1. The neurons were counted and the average count was obtained.

Detection of nerve cell apoptosis by TUNEL assay

TUNEL assay was performed according to the instruction of the kit. After dewaxing, the sections were dehydrated through alcohol gradient (100, 95, 90, 80, 70%) once for 3 min each time. The sections were washed with PBS twice and incubated at 37°C for 30 min with working solution of 20 µg/ml protease K (dissolved in 10 mmol/L Tris/HCL, pH 7.4-8.0). This was followed by washing with PBS, and the surrounding of the sample was dried. Then 50 µl TUNEL reaction mixture was added, and for the negative control, 50 µl labeling solution was added.

The cells were incubated for 60 min at 37°C in the dark and humidified air, and washed with PBS. The apoptotic cells were counted under the fluorescence microscope after adding one drop of PBS (excitation wavelength 450-500 nm, detection wavelength 515-565 nm). PBS was discarded and DAB reagent was added at 50 µl for each slide. The color development reaction was observed under the light microscope for 3 min and then terminated by washing with distilled water. Next the sections were dehydrated through alcohol gradient and transparent with xylene. The slides were sealed with neutral gum. Five non-overlapping visual fields (10×40) were selected at 2 mm to 5 mm from the site of injection (point 0, 2, 5, 7 and 10 with the site of injection as the center of circle). The positive cells were defined as those with nuclei stained brownish yellow. For each slide, 5 representative non-overlapping visual fields (high power) were selected and the average was calculated.

MRNA expressions of Bim and YKL-40 in the hippocampus using RT-PCR

The hippocampus tissues were centrifuged at 4°C for 20 min with supernatant discarded. After washing with PBS three times, total RNA was extracted using Trizol reagent. The extracted RNA was inversely transcribed into cDNA according to the instruction of PCR kit. PCR amplification was performed for Bim, YKL-40 and GAPDH. Primers for Bim: upstream GCC-AAGCAACCTTCTGATGTTA, downstream CAGTG-CCTTCTCCAGACCAG; primers for CHI3L1 encoding YKL-4: upstream 5'-CCAACATCAGCAACAACAAG-3', downstream 5'-CTCCATCCTCCAACAGACAG-3'; the length of the amplified fragment was 124 bp; primers for GAPDH: upstream 5'-CACCTTTGATGCTGGGGCT', downstream 5'-GGTCCAGGGTTTCTTACTC-3'; the length of the amplified fragment was 130 bp. PCR reaction: denaturation at 95°C for 3 min, denaturation at 94°C for 10 s, annealing at 55°C for 30

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Table 4. Comparison of effective time of exploration in the quadrants of water maze, percentage of swim distance to total distance and number of times of passing the platform

Group	Effective time	Percentage of swim distance to total distance	Number of times of passing the platform
Control group	8.12±1.65	43.15±1.13	5.76±1.28
SE group	4.60±1.58*	25.43±1.51*	2.89±1.40*
Hyperbaric oxygen therapy group	6.16±1.32*, ^a	37.03±1.67*, ^a	4.91±1.37*, ^a

Note: *P<0.05 compared with the control; *,^aP<0.01 compared with the control; ^aP<0.05 compared with SE group.

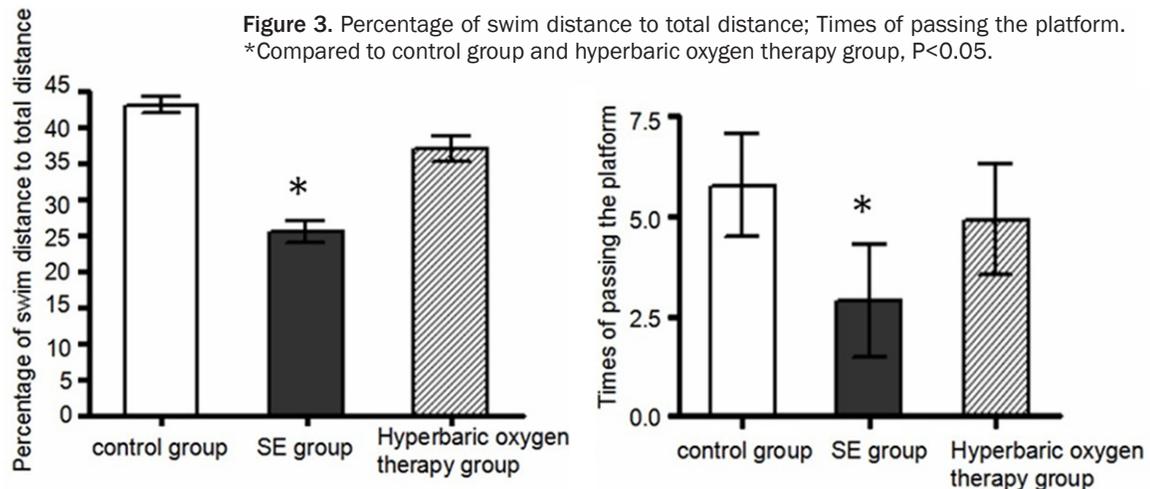


Figure 3. Percentage of swim distance to total distance; Times of passing the platform. *Compared to control group and hyperbaric oxygen therapy group, P<0.05.

Table 5. Comparison of the number of YKL-40-positive cells between the groups

Group	3 d	4 d
Control group	0.00±0.00	0.00±0.00
SE group	21.38±1.32*	15.13±1.27*
Hyperbaric oxygen therapy group	14.21±1.30*	9.86±1.24*

Note: *P<0.05.

s, extension at 72°C for 30 s, 40 cycles, final extension at 72°C for 7 min. The products were preserved at 4°C and analyzed by 2% agarose gel electrophoresis. The scanned gels were analyzed using gel image analysis system. The ratio of the absorbance of Bim and YKL-40 to that of GAPDH was calculated as the relative intensity of mRNA expression.

Protein expressions of bim and YKL-40 in the hippocampus using Western blotting detection

The frozen hippocampus tissues were taken out and weighed, and cut into pieces using tissue scissors. Then the tissues were loaded to the cryogenic tube and placed on ice. The cells were added with 1 µl PMSF lysis buffer, shaken

well and placed on ice for 30 min. The proteins were extracted and diluted to 0.5 mg/ml. The protein concentration was detected with the Bradford technique. Electrophoresis was performed at constant voltage of 40 V for 1 h using 5% stacking gel and then at the constant voltage of 60 V for 3.5 h using 10% separating gel. Finally tank transfer

was performed at the constant voltage of 14 V for 14 h, followed by incubation on a shaker at 37°C for 2 h. Then the cells were incubated with rabbit anti-rat monoclonal antibody against YKL-40 (diluted in TBST, 1:1000) and rabbit anti-rat polyclonal antibody against GAPDH (diluted in TBST, 1:1000) at 4°C overnight in a wet box. Then the sections were washed with PBS three times for 5 min each time. Incubation on a shaker was carried out at 37°C for 2 h by adding the corresponding goat anti-rat biotinylated secondary antibody IgG (1:1000). After that, the membranes were washed with TBST three times for 10 min each time. Then goat anti-rat biotinylated secondary antibody IgG (1:500) was added and the cells were incubated on a shaker at 37°C for 60 min.

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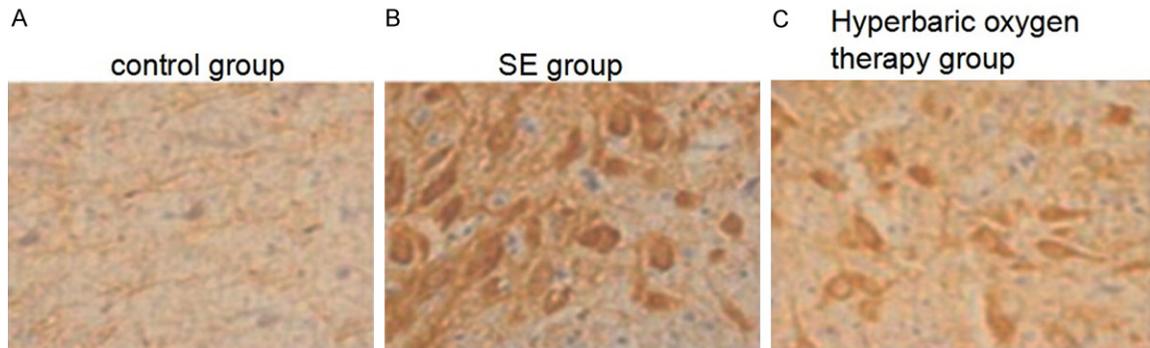


Figure 4. Comparison of the number of YKL-40-positive cells.

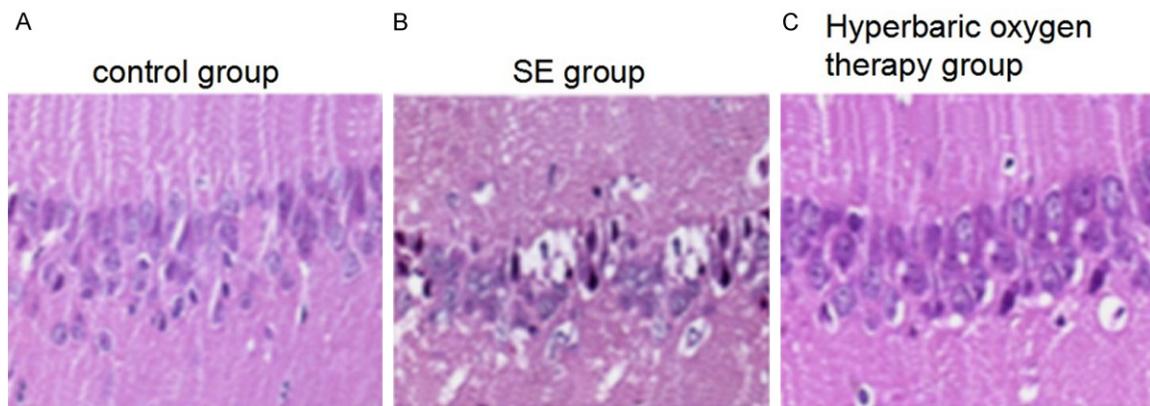


Figure 5. A: Normal morphology and number of neurons in the hippocampus in the control group ($\times 400$); B: Obviously reduced neuron number in the hippocampus in the SE group, with balloon-like neurons and apoptotic bodies ($\times 400$); C: Increased neuron number with basically normal morphology ($\times 400$).

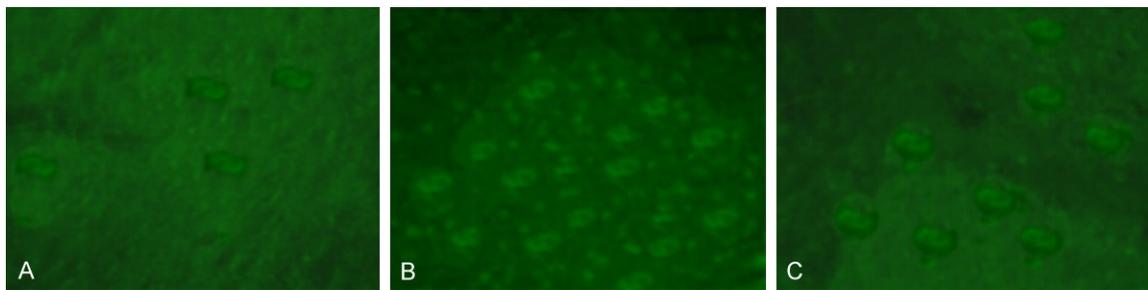


Figure 6. A: Number of Tunel-positive cells in the control group (fluorescence $\times 100$); B: Number of Tunel-positive cells in the SE group (fluorescence $\times 100$); C: Number of Tunel-positive cells in the hyperbaric oxygen therapy group (fluorescence $\times 100$).

The membranes were washed with TBST four times for 5 min each time, and DAB reagent was added. This procedure was repeated three times before image analysis using Quantity One software. The ratio of the integral absorbance between the target gene and β -actin was calculated to express the relative protein expression.

Statistical process

The statistical analysis was carried out using SPSS 15.0 software, and analysis of variance was carried out for completely randomized design. The data were reported as mean \pm standard deviation ($\bar{x} \pm s$). Multiple comparison procedure was performed for all treatments

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Table 6. Comparison of the number of apoptotic cells in the brain tissues after injection of anti-brain antibodies between the three groups ($\bar{x} \pm s$)

Group	Number of rats	Number of positive cells in TUNEL assay
SE group	0.00±0.00	0.00±0.00
Hyperbaric oxygen therapy group	21.38±1.32*	15.13±1.27*
Control group	14.21±1.30*	9.86±1.24*

Note: *P<0.05.

Table 7. mRNA expressions of Bim and YKL-40 in each group

Gen	Control group	SE group	Hyperbaric oxygen therapy group
Bim	0.00±0.00	1.86±0.58	0.46±0.23
YKL-40	0.00±0.00*	1.77±0.24*	0.35±0.25*

Note: *P<0.05.

using Dunnett's test. P<0.05 indicated significant difference.

Results

Results of behavioral observation

Comparison of the onset number of spontaneous seizures: After awakening from anesthesia with chloral hydrate, the spontaneous seizures lasted for about 20 h (20.46±0.33) for rats in the SE group; the severity of the seizures was alleviated in the hyperbaric oxygen therapy group, and the seizures were intermittent and paroxysmal; the seizures lasted for 2-3 h (1.23±0.24) and then stopped spontaneously. The duration of seizures was obviously shorter in the hyperbaric oxygen therapy group than in the SE group (P<0.05). The rats in the control group had no seizures with normal mental state, behavior and diet. Thus the duration of seizures was 0.00±0.00. The difference between the hyperbaric oxygen therapy group and the control group was not statistically significant (P>0.05) (Table 2; Figure 1).

Measurement of spatial learning and memory ability of rats using Morris water maze

The average escaping latency was gradually shortened for each group over the 5 days of navigation ability test. The difference was not significant between the groups on 1 d; the average escaping latency in the hyperbaric oxygen therapy group was obviously shortened on 2 d

compared with the SE group (P<0.05); such difference became more prominent on 3-5 d between the hyperbaric oxygen therapy group and the SE group (P<0.01). During the exploration ability test, the number of times of passing the platform was recorded. Compared with the SE group, the number of times of passing the platform was significantly larger in the hyperbaric oxygen therapy group (P<0.05). The comparison of escaping latency in the navigation ability test between the groups is shown in Table 3 and Figure 2; the comparison of the number of times of passing the platform between the groups is shown in Table 4 and Figure 3. As indicated by the Morris water maze experiment, the rats in the hyperbaric oxygen therapy group displayed an obvious improvement of the spatial learning and memory ability compared with the control group and the SE group (P<0.05).

Detection of YKL-40 positive cells by immunohistochemistry staining

Immunohistochemistry staining identified BrdU-positive cells in hippocampus tissues in both the SE group and the hyperbaric oxygen therapy group. The YKL-40-positive cells were mainly distributed in the pyramidal cell layer of hippocampus region CA3 and CA4 in the affected hemisphere. The number of YKL-40-positive cells in the hyperbaric oxygen therapy group was obviously smaller than that of the SE group on 3 d and 14 d (P<0.05) (Table 5; Figure 4). No YKL-40-positive cells were detected in the control group.

Measurement of number of neurons in the hippocampus region CA1 by HE staining

As found by HE staining, there were more neurons stained in the hippocampus region CA1 in the hyperbaric oxygen therapy group than in the SE group (P<0.05). The neurons in the hippocampus region CA1 in the control group had normal morphology with compact arrangement. The nuclei were round or oval with clear, uniformly stained nucleolus and transparent cytoplasm. Vacuolation was seen in some cells in the hippocampus region CA1 in the SE group; the neurons were arranged disorderly and the neuron number decreased obviously, with

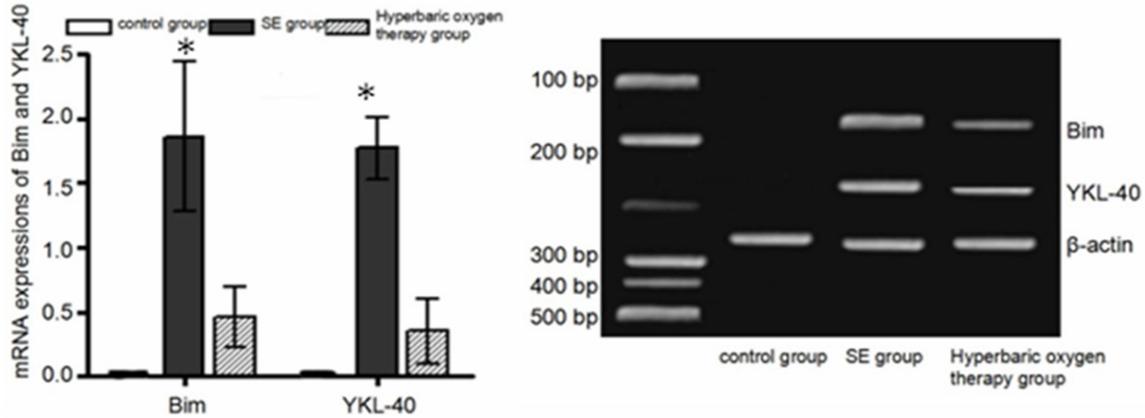


Figure 7. mRNA expressions of Bim and YKL-40 by RT-CPR. *Compared to control group and hyperbaric oxygen therapy group, $P < 0.05$.

Table 8. Protein expressions of Bim and YKL-40

Gen	Control group	SE group	Hyperbaric oxygen therapy group
Bim	0.00±0.00	1.98±0.46	0.38±0.17
YKL-40	0.00±0.00*	1.85±0.37*	0.26±0.23*

Note: * $P < 0.05$.

indistinct cytoplasm. Some neurons showed karyopycnosis, nuclear fragmentation and the presence of apoptotic bodies. Most neurons were normal morphologically in the hyperbaric oxygen therapy group; the neuron number was higher, the neurons were tightly packed, and the nuclei restored the round shape (Figure 5).

Detection of nerve cell apoptosis by TUNEL assay

The apoptotic cells emitted green fluorescence under the fluorescence microscope. A few apoptotic cells were seen in the cortical tissues around the injection site (Figure 6A), and more apoptotic cells were detected in the SE group (Figure 6B). There were fewer apoptotic cells in the cerebral cortex around the injection point in the hyperbaric oxygen therapy group (Figure 6C). The three groups differed significantly in the number of apoptotic cells (Table 6, $P < 0.05$).

Detection of mRNA expressions of Bim and YKL-40 in the hippocampus by RT-PCR

RT-PCR detection showed that the mRNA expressions of Bim and YKL-40 decreased obviously in the hyperbaric oxygen therapy group

compared with the control group and the SE group ($P < 0.05$). The mRNA expressions of Bim and YKL-40 in the hyperbaric oxygen therapy group were significantly different from those of the control group and the SE group ($P < 0.05$). The mRNA expressions of Bim and YKL-40 in each group are shown in Table 7 and Figure 7.

Detection of protein expressions of Bim and YKL-40 in the hippocampus by Western blotting

According to the results of Western Blotting, the protein expressions of Bim and YKL-40 decreased obviously in the hyperbaric oxygen therapy group compared with the control group and the SE group ($P < 0.05$). The protein expressions of Bim and YKL-40 in the hyperbaric oxygen therapy group were significantly different from those of the control group and the SE group ($P < 0.05$). The protein expressions of Bim and YKL-40 in each group are shown in Table 8 and Figure 8.

Discussion

SE is a common and life-threatening neurologic disorder which may cause acute or persistent cognitive and behavioral changes and damage the neurons in the hippocampus [10-12]. If left untreated, SE will cause permanent brain damage. However, we know little about the pathogenesis of the seizures [13-15], and the pathological and physiological mechanism of the brain neuron damage after SE is the topic of heated discussions. Nerve cell apoptosis and loss and glial cell hyperplasia are among the

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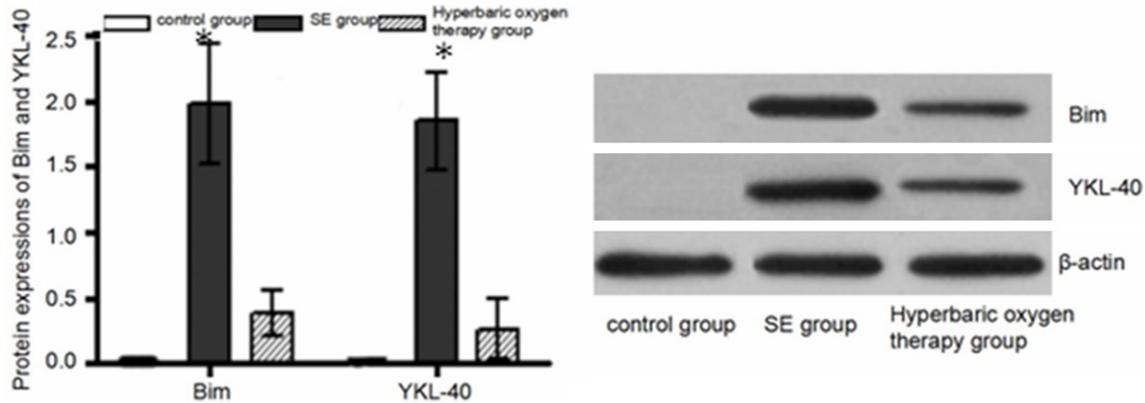


Figure 8. Protein expressions of Bim and YKL-40 by Western Blot. *Compared to control group and hyperbaric oxygen therapy group, $P < 0.05$.

common pathological changes after SE. Cell apoptosis is one reason of brain neuron loss after SE, which in turn plays an important role in the pathology of SE and in neuron death [16-18].

The mechanism of neuron apoptosis after SE is a topic of great interest. Bim, a pro-apoptotic protein containing BH3 domain, is expressed in various tissues. It is found to be associated with the occurrence and progression of tumors and neurologic disorders. Some researchers detected massive expression of Bim in ischemia/reperfusion injury and in the hippocampus with strong pro-apoptotic activity [19, 20]. Studies indicate the importance of immune and inflammatory response to the onset of seizures. YKL-40, an inflammatory protein, has been researched intensively in recent years due to its association with the inflammatory response [21]. YKL-40 gene promotes the inflammatory response and prevents the apoptosis of the inflammatory cells. Since the elevation of YKL-40 level is found to be associated with the severity of some diseases, YKL-40 is considered a potential biomarker of some diseases and the novel target for the treatment of acute and chronic inflammations, cell apoptosis and tumors.

The expression of YKL-40 was detected by immunohistochemistry staining. The results showed that the number of YKL-40-positive cells decreased obviously in the hyperbaric oxygen therapy group as compared with the SE group. The mRNA and protein expressions of Bim and YKL-40 were detected in the hippo-

campus using real-time PCR and Western Blotting, respectively. After hyperbaric oxygen therapy, the Bim and YKL-40 expressions were greatly reduced, with an inhibition of the SE-induced cell apoptosis and inflammatory response according to TUNEL assay. This predicted a better prognosis in SE. The results of behavioral monitoring showed that the onset number of spontaneous seizures reduced remarkably after the hyperbaric oxygen therapy; the spatial learning and memory ability of rats receiving the hyperbaric oxygen therapy was also improved effectively in the Morris water maze experiment. Thus hyperbaric oxygen therapy relieved the symptoms of SE and reduced the frequency of onset.

We believe that hyperbaric oxygen therapy has a positive effect on SE, probably by blocking the cell apoptosis pathway mediated by Bim and YKL-40 gene. Hyperbaric oxygen therapy is free of toxic and side effects and enhances the blood-brain barrier permeability. Thus the anti-epileptic drugs will more easily enter the brain. However, hyperbaric oxygen therapy should not be administered during the major episodes of seizures. Early signs of seizures should be treated timely to avoid onset during the hyperbaric oxygen therapy. Moreover, protective measures should be adopted for patients receiving the hyperbaric oxygen therapy. In our study, hyperbaric oxygen therapy was carried out successfully in all rats. At present, there are much more clinical trials on the application of hyperbaric oxygen therapy in SE [22] than the studies on the mechanism of the protective effect of hyperbaric oxygen therapy. Our

research provides valuable information in this respect. However, due to limitations in the research materials and time, we did not investigate the working mechanism of the hyperbaric oxygen therapy. This will be the topic of further study.

Disclosure of conflict of interest

None.

Address correspondence to: Cheng-Yan Li, Department of Neurology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China. Tel: +86-15222053066; Fax: +86-15222053066; E-mail: lcyancy@163.com

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