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Basic Study

Effect of electroacupuncture at Ganshu (BL 18) and Shenshu (BL 23) on the expression of EphB2 protein in cortex around cerebral infracted area of rat

电针肝俞、肾俞对大鼠脑梗死灶周围皮层 EphB2 蛋白表达的影响

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Abstract

Objective: To observe the effect of electroacupuncture (EA) on the expression of erythropoie-tin-producing hepatocyte receptor B2 (EphB2) in the cortex around the infracted area of middle cerebral artery occlusion (MCAO) rats at different timing, and to reveal the possible mechanism of acupuncture in the treatment of cerebral ischemia**.**

Methods: A total of 180 male Sprague-Dawley (SD) rats were randomly divided into a sham operation group, a model group, an acupoint group and a non-acupoint group, with 45 rats in each group. Rats in each group were further divided into three subgroups: postoperative 3 d, postoperative 14 d and postoperative 21 d groups, with 15 rats in each subgroup. The MCAO model was made by the modified occlusion method. The neurological function score, 2,3,5-triphenyl tetrazolium chloride (TTC) staining, immunohistochemistry assay, immunofluorescence double labeling method and Western blot were used to detect the corresponding indicators.

Results: The neurological impairment of rats was most obvious at postoperative 3 d, and then gradually improved with time, which was more significant in the acupoint group (P<0.05). The change of infarcted volume was consistent with the neurological function impairment. The number of EphB2 positive cells (EphB2$^+$) around the infarcted area was decreased significantly at postoperative 3 d, and then gradually improved with time, which returned to the same level as that in the sham operation group at postoperative 21 d. The increase was most significant in the acupoint group (P<0.05), and the positive cell number was higher than that in the sham operation group (P<0.01). Western blot and immunohistochemistry results were basically consistent. Immunofluorescence displayed that EphB2$^+$ and postsynaptic density-95 positive (PSD-95$^+$) were co-expressed, after the MCAO operation, in the cortical neuron around the infracted area, and the number of co-expressing cells was increased gradually with time, which was most significant in the acupoint group (P<0.05).

Conclusion: Electroacupuncture at Ganshu (BL 18) and Shenshu (BL 23) can significantly improve the neurological function and cerebral infarcted volume ratio of MCAO rats, which may be related to the activation of EphB2 expression in cortex around the infracted area and the promotion of synaptic remodeling.

Keywords: Acupuncture Therapy; Electroacupuncture; Point, Ganshu (BL 18); Point, Shenshu (BL 23); Brain Ischemia; Infarction, Middle Cerebral Artery; Rats

【摘要】目的：观察电针对大脑中动脉梗塞(MCAO) 模型大鼠不同时间点梗死灶周围皮层促红细胞生成素产生肝细胞受体 B2 (EphB2)表达的影响，以期揭示针刺治疗脑缺血病的可能作用机制。**方法：**将 180 只雄性 Sprague-Dawley (SD)大鼠随机分为假手术组、模型组、穴位组和非穴位组，每组 45 只；各组又分为术后 3 d, 14 d 及 21 d 三个亚组，每组 15 只。采用改良线栓法复制 MCAO 大鼠模型，采用神经功能评分、2,3,5-氯化三苯基四氮唑(TTC)染色、免疫组化法、免疫荧光双标法及 Western blot 法进行相应指标检测。**结果：**大鼠术后神经功能缺损在 3 d 时最明显，之后随着时间延长逐渐改善，其中以穴位组更显著(P＜0.05)；且梗死灶体积变化与神经功能缺损基本一致。术后 3 d 梗死灶周围 EphB2 阳性(EphB2$^+$)细胞数明显减少，随着时间推移表达逐渐增加,至 21 d 恢复至假手术组水平，其中以穴位组增加最明显(P＜0.05)，且阳性细胞数高于假手术组(P＜0.01)。Western blot 与免疫组化

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检测结果基本一致。免疫荧光显示大鼠术后 EphB2$^+$与突触后致密物-95 阳性(PSD-95$^+$)在梗死灶周围皮层神经细胞上共表达，且随时间延长共表达细胞数逐渐增加，以穴位组增加最明显($P<0.05$)。**结论：**电针肝俞、肾俞能明显改善 MCAO 大鼠神经功能及脑梗死体积比，可能与激活梗死灶周围皮层 EphB2 表达，促进突触重塑有关。
【关键词】针刺疗法；电针；穴，肝俞；穴，肾俞；脑缺血；大脑中动脉梗塞；大鼠
【中图分类号】R2-03　　**【文献标志码】**A

The central nervous system (CNS) has the ability to repair its own structure and function, which mainly based on the plasticity potential of the brain at different levels of the synaptic and neural network, synaptic remodeling is the most important. The peripheral axons of the uninfected neurons appears budding after the cerebral infarction and form a new collection with the infected neurons, which provides an important material basis for neurological rehabilitation[1]. Synaptic regeneration depends on the mutual contact between cells, and the axon-dendritic docking triggers new establishment of presynaptic and postsynaptic structure[2]. Adhesion molecules that mediate cell-cell contact are critical in the process of synaptic remodeling. Erythropoie-tin-producing hepatocyte receptor B2 (EphB2) is a adhesion molecule among synapses, the PDZ-binding domain at its end can bind to postsynaptic density protein 95 (PSD-95), and then recruit a large number of protein complexes to form the new presynaptic and postsynaptic structure[2]. Studies showed that EphB2 involved in embryonic neural axonal development, regulation process of synaptic plasticity[3]; and electroacupuncture (EA) could promote nerve regeneration[4]. The purpose of this study was to observe the effect of EA on EphB2, and to explore the pathways and possible mechanisms of EA treatment of cerebral ischemia.

1 Material and Methods

1.1 Experimental animals and groups

A total of 180 SPF grade male adult Sprague-Dawley (SD) rats, weighing (250±20) g, were provided by the Experimental Animal Center of Hunan University of Chinese Medicine [certificate number: SYXK (Hunan)-2013-0005]. The 180 rats were randomly divided into a sham operation group (SOG), a model group (MG), an acupoint group (AG) and a non-acupoint group (NAG), according to the random number table, 45 rats in each group.

Rats in each group were further divided into 3 subgroups of a postoperative 3 d group, a postoperative 14 d group and a postoperative 21 d group, 15 rats in each subgroup. The rats were treated in accordance with the *Guideline Opinion of the Ministry of Science and Technology on the Treatment of Experimental Animals* in 2006.

1.2 Main reagents and instruments

EphB2 (SC-28980, Santa Cruz Biotechnology, Inc., USA), HL-25142 Neofuge 15R refrigerated centrifuge (Shanghai Lishen Scientific Instrument Co., Ltd., China), RM2016 slicing machine (Shanghai Leica Instrument Co., Ltd., China), filliform needle of 0.30 mm in diameter and 25 mm in length (batch number: 130291, Suzhou Medical Products Factory Co., Ltd., China), 20100114 G6805-Ⅱ EA apparatus (Qingdao Xin Sheng Industrial Co., Ltd., China).

1.3 Model preparation and treatment

The right middle cerebral artery occlusion (MCAO) model was prepared according to the modified method of MCAO[5]. The model scores were conducted when the rats were awake from anesthesia. The 5-point scale developed by Longa EZ, *et al* was used to evaluate whether the models were successful[6]: rats with 1-3 points were included in the following experiments, and the others were excluded. The intervention method in each group was as follows.

SOG: Rats in the SOG were ligated at the common carotid artery without occlusion. During the experiment, rats accepted binding without treatment.

MG: Rats in the MG accepted binding without treatment.

AG: Rats in the AG were subjected to the EA treatment at Ganshu (BL 18) and Shenshu (BL 23) after the binding. The acupoint locations were determined by anthropomorphic comparison positioning method recorded in the *Experimental Acupuncture Science*[7]. Ganshu (BL 18): 5 mm below the 9th thoracic vertebrae; Shenshu (BL 23): 5 mm below the second lumbar vertebrae. Perpendicular needling was conducted at the acupoints for 6 mm and then connected to EA apparatus. An output electrode was connected to both acupoints at the same side. Stimulation parameters: the density wave of 2 Hz/30 Hz, the current intensity of 2 mA, the output voltage of 2-4 V, and the degree of local gentle fibrillation.

NAG: locations at 1 cm away from the two acupoints of Ganshu (BL 18) and Shenshu (BL 23) were selected as the non-acupoint controls after the routine binding treatment of rats in the NAG, and subjected to the EA treatment with the same stimulation parameters used in the AG.

The intervention started on the first day after the modeling was successful in each group. Each stimulation

lasted 30 min, once a day. A total of 10 rats in each group were randomly selected after 3 d, 14 d or 21 d EA treatment, and the neurological function scores were evaluated according to the method recorded in the *Animal Experimental Methodology*[8]. The higher the score, the more serious the neurological function injury.

1.4 Determination of cerebral infarcted volume

After continuous treatments for 3 d, 14 d or 21 d, 5 rats in each group were randomly selected for cerebral infarcted volume measurement. Five frozen sections were sliced continuously into slices of 2 mm in thickness for each brain tissue, stained completely with 2,3,5-triphenyl tetrazolium chloride (TTC), fixed with formaldehyde and taken pictures. The infarcted volume ratio was calculated by Image-Pro-Plus 6.0 software. The infarcted volume ratio = (Cerebral hemisphere volume in the normal side − Cerebral hemisphere volume of noninfarcted area in the infarcted side) ÷ Cerebral hemisphere volume in the normal side × 100%.

1.5 Items detection

After 3 d, 14 d or 21 d of continuous treatments, 5 rats in each group were randomly selected for immune-histochemistry and immunofluorescence double labeling.

1.5.1 Immunohistochemical staining

The rat brain tissues were separated after fixed with paraformaldehyde and embedded for the sections. The sections were sealed after immunohistochemical staining. Five high magnification fields (×400) from the same part of the infarcted area of each rat were randomly selected and taken pictures using the BX51T-PHD-J11 microscope collecting and analyzing system. The same brown-yellow color was selected by Image-Pro-Plus 6.0 as the unified standard to determine the positive cells of all the pictures. The integral optical density (IOD) of the positive expression region was calculated. The positive cells were those with brown stained cytoplasm.

1.5.2 Immunofluorescence double labeling

The brain tissues of the rats were separated, fixed with paraformaldehyde and embedded. The sections were sliced, stained by immunofluorescence double labeling and observed under a Nikon inverted fluorescence microscope to obtain the images.

1.5.3 Western blot procedures

After 3 d, 14 d or 21 d of continuous treatments, 5 rats in each group were randomly selected and used for Western blot analysis. Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate [400 mg/(kg·bw)], and then sacrificed by dislocation to separate the right brain tissues. Total proteins were extracted from the right brain tissues of the rats by homogenization and subjected to Western blot analysis. The films were developed and scanned for archive. The Alpha software processing system was used to analyze the gray values of the target bands. Relative expression of each protein to be tested = Gray value of the protein to be tested ÷ Gray value of β-actin.

1.6 Statistical analysis

The data were analyzed by the SPSS 19.0 version statistical software. Data fitting the normal distribution were presented as mean ± standard deviation ($\bar{x} \pm s$). Data not fitting the normal distribution were presented as mean ± 95% confidence intervals. One-way ANOVA was used to compare the difference among the groups, and least significant difference (LSD) was used to compare the difference between two groups. $P<0.05$ indicated the difference was statistically significant.

2 Results

2.1 Behavioral observation

The difference of Zea-Longa score was statistically significant ($P<0.01$) between the MG and the SOG 2 h after the operation, indicating that the model was successfully prepared; there was no significant difference among the modeled groups ($P>0.05$), indicating comparability (Figure 1).

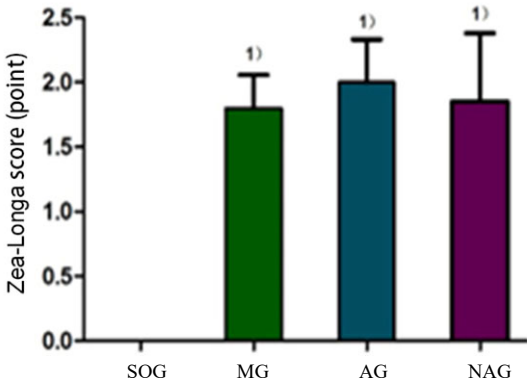

Figure 1. Comparison of Zea-Longa score 2 h after modeling

Note: Compared with the SOG, 1) $P<0.01$

Rat's neurological function was evaluated according to the evaluation criteria of neurological function in the *Experimental Acupuncture Science*[7]. The scores of the 3 subgroups were the highest on the postoperative day 3, and then were decreased on postoperative day 14 and day 21. There was a significant difference between postoperative day 3 and day 21 ($P<0.05$). Score of each timing in the AG was significantly lower than that in the MG ($P<0.01$) and NAG ($P<0.05$). Only neurological function score in the NAG on postoperative day 21 was slightly lower than that in the MG, but the difference was statistically insignificant ($P>0.05$), (Table 1).

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Table 1. Comprising neurological function score of each group after MCAO operation（$\bar{x}\pm s$, point）

Group	*n*	Postoperative 2 h	Postoperative day 3	Postoperative day 14	Postoperative day 21
SOG	10	0±0	0±0	0±0	0±0
MG	10	7.40±0.94	7.80±0.92	7.40±0.84	6.60±0.97[2)]
AG	10	7.10±0.86	6.30±1.06[4)5)]	4.90±1.10[1)4)6)]	3.60±1.58[2)3)4)6)]
NAG	10	7.30±1.03	7.50±1.27	6.50±1.08	5.60±0.97[2)]

Note: Compared with postoperative day 3 in the same group, 1) $P<0.05$, 2) $P<0.01$; compared with postoperative day14 in the same group, 3) $P<0.01$; compared with the MG at the same timing, 4) $P<0.01$; compared with the NAG at the same timing, 5) $P<0.05$, 6) $P<0.01$

2.2 TTC staining

TTC staining all showed red (not infarcted area) in the SOG. Infarcted locations of the MG were white, mainly in the right cerebral cortex and basal ganglia (Figure 2). The infarcted volume ratios of the brain tissue in the MG, AG and NAG were shown in Table 2.

The infarcted volume was the largest on postoperative day 3, and the infarcted volume was gradually decreased on postoperative day 14 and postoperative day 21; compared with the MG and the NAG, the decrease of cerebral infarcted volume was the most significant in the AG at each timing (all $P<0.05$), however, there was no significant difference between the MG and the NAG ($P>0.05$), (Table 2).

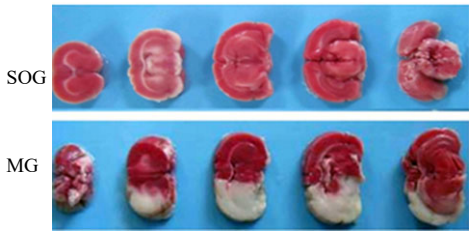

Figure 2. TTC staining picture

Table 2. Comparing the infarcted volume among the groups ($\bar{x}\pm s$, %)

Group	*n*	Postoperative day 3	Postoperative day 14	Postoperative day 21
SOG	5	0±0	0±0	0±0
MG	5	30.40±1.60	21.04±2.24[1)]	14.78±2.35[1)]
AG	5	27.00±1.26[3) 4)]	16.88±1.02[1)3)4)]	9.60±1.23[1)2)3)4)]
NAG	5	29.35±1.35	19.89±2.39	13.05±1.94[1)]

Note: Compared with postoperative day 3 in the same group, 1) $P<0.01$; compared with postoperative day 14 in the same group, 2) $P<0.01$; compared with the same timing in the MG, 3) $P<0.01$; compared with the NAG, 4) $P<0.01$

2.3 Expression of EphB2 in the cerebral cortex around the infarcted area

EphB2 was expressed in the cytoplasm and cell membrane of the neurons surrounding the infarcted area, and the positive cells appeared yellow brown. Compared with the SOG, EphB2 positive expression ($EphB2^+$) around the infarcted area was significantly decreased in the MG on postoperative day 3, which had been restored to some extent at postoperative day 14 and day 21, while still less than that in SOG. $EphB2^+$ expression around the infarcted area was significantly increased in the AG at postoperative day 14, and there was no significant difference compared with that in the SOG at postoperative 21 d ($P>0.05$); and $EphB2^+$ expressions in the AG were all more than that in the NAG at each timing ($P>0.05$). There was no statistically significant difference in the $EphB2^+$ expression between the NAG and the MG ($P>0.05$), (Figure 3 and Figure 4). The above-mentioned differences of $EphB2^+$ expression were further confirmed by Western blot analysis (Figure 5).

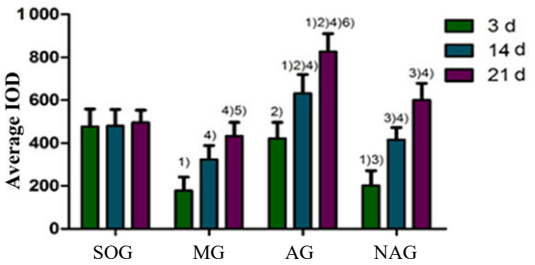

Figure 3. Comparing immunohistochemical expression of EphB2 in the infarcted area after MCAO

Note: Compared with the SOG at the same timing, 1) $P<0.01$; compared with the MG, 2) $P<0.01$; compared with the NAG, 3) $P<0.01$; compared with postoperative day 3 in the same group, 4) $P<0.01$; compared with postoperative day 14 in the same group, 5) $P<0.05$, 6) $P<0.01$

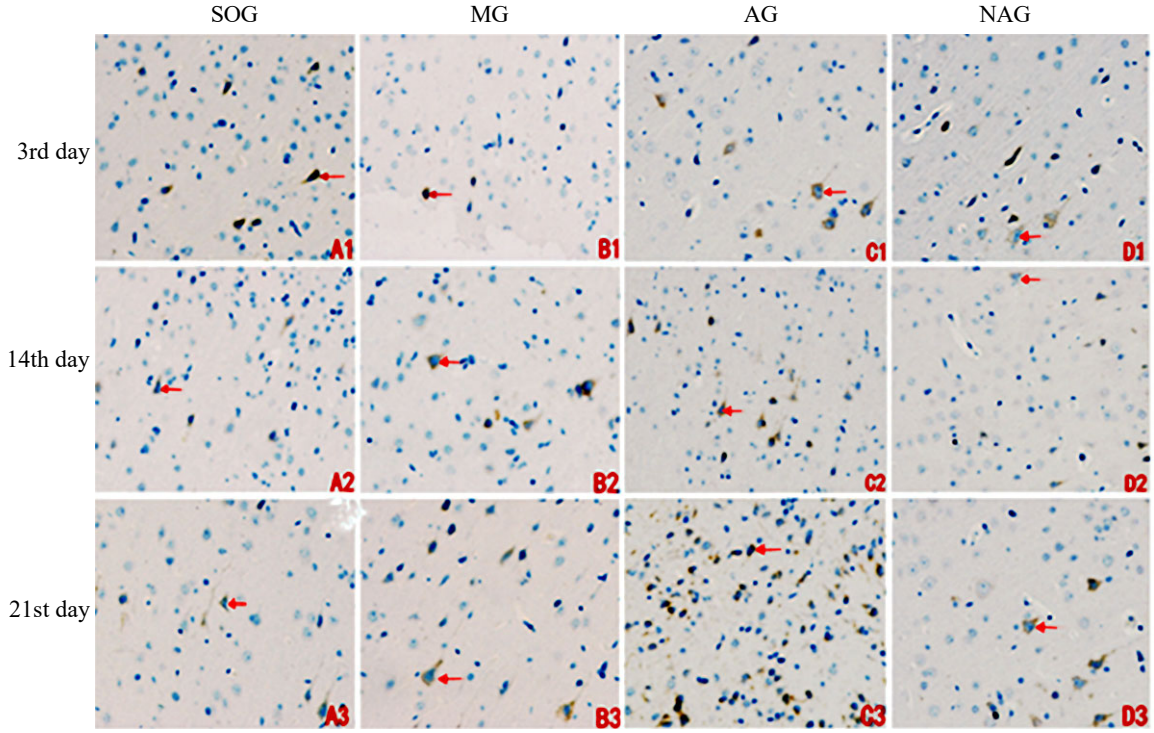

Figure 4. Expression of EphB2 around the infarcted area after MCAO in each group (×400)

Note: Red arrow indicates EphB2$^+$

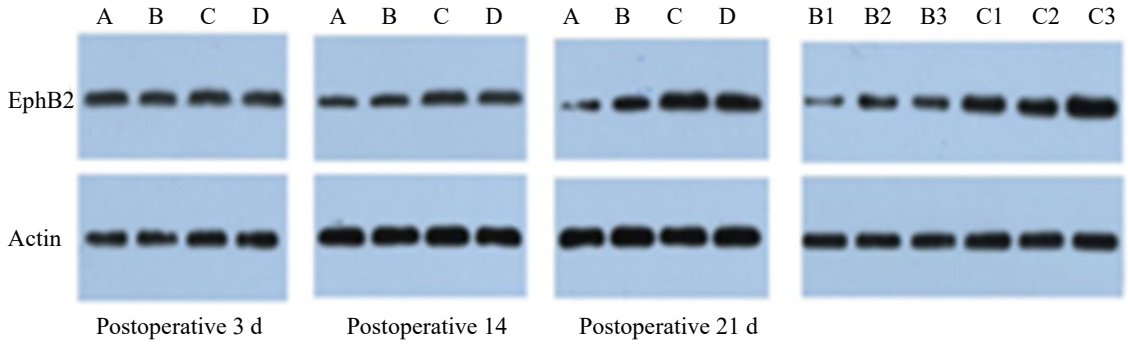

Figure 5. EphB2 expression around the infarcted area at different timing after MCAO by Western blot analysis

Note: A=SOG; B=MG; C=AG; D=NAG; B1=MG postoperative day 3; B2=MG postoperative day 14; B3=MG postoperative day 21; C1=AG postoperative day 3; C2=AG postoperative day 14; C3=AG postoperative day 21

2.4 The positive expression of EphB2 in the cerebral cortex around the infarcted area/postsynaptic density-95 (PSD-95)

A small amount of PSD-95$^+$ cells were found around the infarcted area after MCAO. After EA treatment at Ganshu (BL 18) and Shenshu (BL 23), PSD-95$^+$ cells were significantly increased, and the fluorescence signal overlaps of EphB2$^+$/PSD-95$^+$ were significantly increased. The number of EphB2$^+$/PSD-95$^+$ cells around the infarcted area in the AG 3 d after MCAO showed no statistical significant difference with the SOG (all $P>0.05$), but higher than those in the MG and NAG (all $P<0.05$) . The number of EphB2$^+$/PSD-95$^+$ cells around the infarcted area in the AG was significantly higher than that in other groups on postoperative day 14 and day 21 (both $P<0.05$). Compared with the SOG, EphB2$^+$/PSD-95$^+$ cell numbers in the MG and NAG onpostoperative day 21 showed no statistically significant differences (both $P>0.05$), (Figure 6 and Figure 7).

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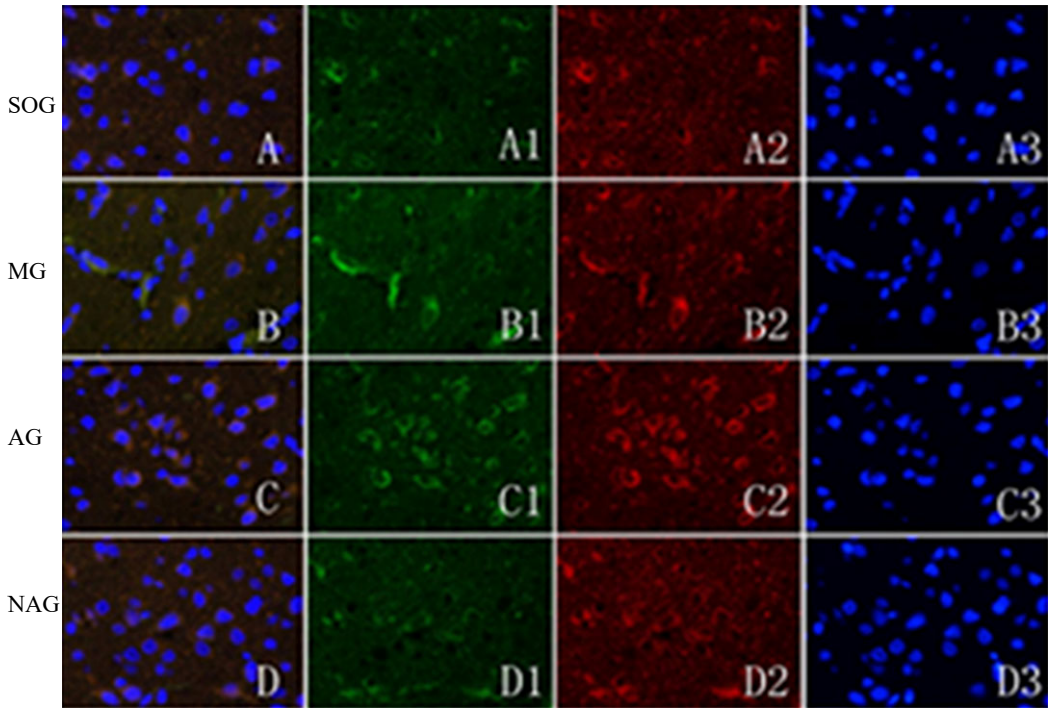

Figure 6. EphB2$^+$/PSD-95$^+$ fluorescence double labeling pictures on day 21 after MCAO in each group (×400)

Note: EphB2$^+$ expression (green), PSD-95$^+$ expression (red), nucleus was blue. A, B, C or D was the overlap of the three colors mentioned above, respectively

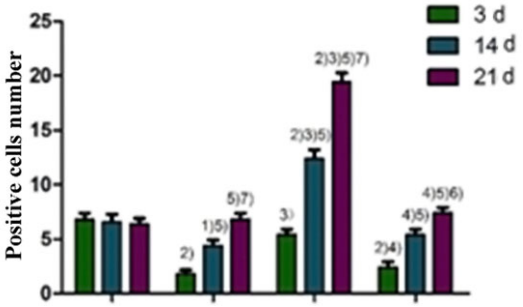

Figure 7. The number of positive cells of EphB2$^+$/PSD-95$^+$ fluorescence double labeling around the infracted area in each group after MCAO

Note: Compared with the SOG at the same timing, 1) P<0.05, 2) P<0.01; compared with the MG at the same timing, 3) P<0.01; compared with the AG at the same timing, 4) P<0.01; compared with the postoperative day 3 in the same group, 5) P<0.01; compared with the postoperative day 14 in the same group, 6) P<0.05, 7) P<0.01

3 Discussion

3.1 Selection of the acupuncture protocol

The root cause of stroke is deficiency, mainly the yin deficiency of the liver and kidney, however, patients often present with excessive symptoms. Therefore, the treatment should focus on regulation and tonification of the liver and kidney. Ganshu (BL 18) and Shenshu (BL 23) selected for EA stimulation in this experiment were mainly based on the following four reasons: first, Ganshu (BL 18) and Shenshu (BL 23) belong to the Bladder Meridian which passes the head and enters the brain; Ganshu (BL 18) and Shenshu (BL 23) are the door for the qi of the liver and kidney to be infused into the Meridian of Foot Taiyang. Second, the Bladder Meridian plays an important role in the function of the brain in governing spirit, connecting with the brain, and intersects with the Governor Vessel on the top of the head, and has the role to nourish the primordial spirit. Third, the liver stores blood to produce essence and the kidney stores essence to produce marrow. Since essence and blood share the same source, and the brain is the sea of marrow, if the sea of marrow has disorder, the root disorder should be in the liver and kidney, therefore, liver and kidney should be treated. Ganshu (BL 18) and Shenshu (BL 23) were selected based on the pathogenesis of stroke to unblock the meridians and reinforce the liver and kidney to tonify essence and marrow, benefit the brain, disperse stasis, clear collaterals, and open orifices. Fourth, according to the modern medicine, Ganshu (BL 18) and Shenshu (BL 23) locate at the body surface projection area of the sympathetic trunk in the chest/abdomen and the spinal cord, where the spinal nerve passes. Acupuncture at Ganshu (BL 18) and Shenshu (BL 23) both affects the sympathetic nerve and regulates the autonomic nerve function and motor nerve function[9]. Experiments showed that[10], in the acute phase of cerebral ischemia in rats, EA stimulation could down-regulate the expression of neurocan mRNA, and improve behavioral

scoring. The therapeutic effect of EA at Ganshu (BL 18) and Shenshu (BL 23) was better.

In the previous study, we found that EA at Ganshu (BL 18) and Shenshu (BL 23) could increase the density of Ⅲ-Ⅳ layer neurons in hippocampal CA1 area and the cortex, reduce cerebral infarcted volume and improve the behavioral score[11]. Therefore, in this study we selected Ganshu (BL 18) and Shenshu (BL 23) for EA stimulation.

3.2 Effect of acupuncture on neurological function recovery in MCAO rat models

Cerebral infarcted volume ratio and neurological function score are the most intuitive indicators to evaluate the degree of ischemic brain injure. Most studies showed that the infarcted volume of MCAO rats peaked on postoperative day 3, and then was gradually decreased with time[12]. The results of this study were consistent with the reported studies, indicating that cerebral ischemia rat showed a certain degree of self-recovery ability. Compared with the MG and the NAG, decrease of the cerebral infracted volume was most significant in the AG, which may be related to the specific effect of EA at Ganshu (BL 18) and Shenshu (BL 23) to activate the endogenous repair mechanism of brain. The postoperative neurological function scores between the MG and the SOG were statistically different. The neurological function score of the MG was gradually decreased with time. This self-repairing ability further validated the results of the cerebral infarcted volume ratio.

3.3 Effect of acupuncture on the expression of EphB2 in neurons of MCAO model rats around the infracted area

Synaptic remodeling is the most important ability of brain plasticity. Disappearance of synaptic activity is the earliest performance of the cerebral ischemia. The results showed that the cerebral plasticity was the most obvious one month after cerebral infarction, and the recovery of nerve function was also the fastest. The self-repair of the CNS depends on synaptic remodeling, and the development of synapses depends on cell-cell contact[2], and adhesion molecules are important in synaptic development[13]. As an adhesion molecule, EphB2 is one of the most important members of the receptor tyrosine kinase (RTK) family, which is known to play an important role in enhancing the motor ability of dendritic spine pseudopodia and promoting axonal growth and regeneration[14].

Our current results showed that $EphB2^+$ around the infracted area in the MG was significantly lower than that in the SOG on postoperative day 3, and the expression of EphB2 was gradually increased in the MG, and reached the same level as in SOG on postoperative day 21. It is reported that the cerebral cortex after cerebral infarction will experience cell death, inflammation, gliosis, oxidative stress and neural activity decrease due to cortical inhibition[15]. Axonal regeneration and synaptic formation around the infracted area occurred 1-3 d after the infarction, and continued to develop during 7-14 d and formed the mature synapse around the infracted area on postoperative day 28[16]. In the current study, we observed that the change mode of EphB2 expression with time around the infarcted area was coincided with the change of synaptic remodeling after infarction described above.

To further demonstrate the relationship between EphB2 and synaptic remodeling, we selected the post-synaptic membrane-specific marker PSD-95 as an indicator of synaptic regeneration. PSD-95, a member of the guanylate kinase family, possesses a synaptic scaffold protein and a complex protein-protein interaction region. It can not only bind to N-methyl-D-aspartate receptors but also the related proteins in the signal pathways to form the receptor-signaling molecule-regulatory molecule-target molecule complex and participate in the formation and maintenance of synaptic connections through the interaction of multiple proteins of PSD[17]. The PDZ binding domain of the EphB2 receptor can bind to PSD-95 and recruit a large amount of protein complex to form a new presynaptic and postsynaptic structure[2]. In the present study, EphB2/PSD-95 immuno-fluorescence double labeling showed that the number of positive cells in each group increased gradually, the increase in the AG was the most significant, indicating that EA at Ganshu (BL 18) and Shenshu (BL 23) could enhance the $EphB2^+$/$PSD\text{-}95^+$ co-expression around the infracted area. We further confirmed that EphB2 was involved in axon regeneration and synaptic formation after infarction[18], which has been reported before. Kayser MS, *et al*[19] found that EphB2 receptor was activated in neurons, cultured in vitro, by ephrinB/Fc extracellular fusion protein ion. EphB2 directly binds and activates N-methyl-D-aspartate receptor to increase synthesis of new proteins, so that the number of synapses of glutamic acid energy increased. EA stimulation could promote the expression of $EphB2^+$ and change the synaptic structure of hippocampal pyramidal neurons; in contrast, attenuated NMDA-mediated synaptic currents were recorded on the hippocampal dentate gyrus (DG) neurons of mice with EphB2 gene knocked out; suggesting that EphB2 plays an important role in the regulation of synaptic structure and function. Therefore, in the current study, $EphB2^+$ around the infracted area on postoperative day 3 decreased, which may be associated with neuron death and inhibitory strengthen of nerve activity at early infarction period.

With the axonal regeneration and synapse formation, which were gradually increased during postoperative day 14-21, $EphB2^+$ around the infracted

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area was also gradually increased, especially in the AG, and was significantly higher than that in the other groups on postoperative day 21. The above-mentioned changes of EA at Ganshu (BL 18) and Shenshu (BL 23) simultaneously had a good correlation with neurological function score and cerebral infarcted volume ratio. This ultimately confirmed that EphB2 was involved in the post-infarction synaptic remodeling, and acupuncture had a synergistic effect on synaptic remodeling.

EA could reduce cerebral infarcted volume and neurological function score of MCAO rats. Combined with the results of this study, the mechanism of EA treatment of cerebral ischemia may be related to its regulation of EphB2 expression around the infracted area, and thus promote axonal regeneration and synaptic formation around the infracted area; however, further studies are necessary to investigate how EA can specifically interfere with the signal transduction of synaptic remodeling to achieve the treatment of cerebral ischemia.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

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