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Transcutaneous Electrical Acupoint Stimulation Promotes PGC-1α Mediated Mitochondrial Biogenesis and Antioxidant Stress to Protect Cognitive Function in Vascular Dementia Rats*

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Abstract **Objective** The purpose of this study was to investigate the effects of transcutaneous electrical acupoint stimulation (TEAS) on cognitive function of vascular dementia (VD) rats and its mechanism. **Methods** VD rat model was established by modified two-vessel occlusion (2-VO). After modeling, TEAS and electroacupuncture (EA) were used to stimulate Baihui and Zusanli points of rats respectively for 14 d. After treatment, novel object recognition test, Morris water maze test, and Y maze test were used to evaluate the spatial memory and learning ability of rats. Hematoxylin and eosin staining was used to observe the morphology of hippocampal neurons. Transmission electron microscopy was used to observe the ultrastructure of hippocampal mitochondria. Enzyme-linked immunosorbent assay kits were used to detected the levels of SOD, CAT, GSH-Px, MDA and ROS in serum of rats. Western blot was used to detect the expression of PGC-1α, TFAM, HO-1, NQO1 proteins in the hippocampus, Keap1 protein in the cytoplasm and Nrf2, NRF1 proteins in the nucleus. **Results** After treatment for 14 d, compared to the model group, the escape latency of VD rats decreased, while the discrimination index, the times of rats crossing the original platform area, the residence time in the original platform quadrant, and the percentage of alternation increased. TEAS can improve the structure of hippocampal neurons and mitochondria of VD rats, showing that neurons were arranged more regularly and distributed more evenly, nuclear membrane and nucleoli were clearer, and mitochondrial swelling were reduced, mitochondrial matrix density were increased, and mitochondrial cristae were more obvious. The levels of SOD, GSH-Px and CAT in serum increased significantly, while the concentration of MDA and ROS decreased. TEAS also up-regulated the expression levels of PGC-1α TFAM, NQO1 and HO-1 proteins in the hippocampus and Nrf2, NRF1 proteins in the nucleus, but down-regulated the Keap1 protein in the cytoplasm. **Conclusion** TEAS can improve cognition, hippocampal neurons and mitochondrial structure of VD rats, and the effect is better

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than EA. The mechanism may be the activation of PGC-1α mediated mitochondrial biogenesis and antioxidant stress, which also provides a potential therapeutic technology and experimental basis for the treatment of VD.

Key words vascular dementia, transcutaneous electrical acupoint stimulation, cognition function, PGC-1α, anti-oxidant, mitochondrial

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Vascular dementia (VD) is cognitive dysfunction due to cerebrovascular disease. Its incidence is second only to Alzheimer's disease (AD)[1]. In recent years, the incidence of cerebrovascular diseases has been increasing, which bring great burden to oneself, family and society[2]. Because the pathogenesis of VD is still unclear, there is still a lack of effective treatment or drugs.

Chronic cerebral hypoperfusion is one of the main causes of VD, and its pathological process is the result of the interaction of multiple complex mechanisms, including oxidative stress, inflammatory, mitochondrial dysfunction, excitatory toxicity, Ca^{2+} overload and apoptosis[3]. The increase of oxygen free radicals caused by oxidative stress is the primary link of neuron damage, and mitochondrial dysfunction plays a bridge role in VD[4]. Studies have shown that the hippocampal and cortical neurons in VD rat are vulnerable to the influences of hypoxic ischemic factors that led to its structure and function of mitochondria abnormalities[5-6]. The above results further lead to severe brain energy deficiency and the release of a large number of reactive oxygen species (ROS), resulting in the imbalance between brain oxidation and anti-oxidant defense mechanisms[7-9]. Therefore, maintaining the integrity of mitochondrial structure and function in order to enhance the tolerance of brain regions closely related to memory to ischemic hypoxia stimulation is of great significance for the treatment of VD.

Peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α) is an important transcription coactivator regulating mitochondrial biogenesis. It is highly expressed in neural tissues. Studies have found that, PGC-1α can activate the transcription of nuclear respiratory factor (NRFs), estrogen related receptorα (ERRα) and peroxisome proliferator-activated receptors (PPARs), and then regulate mitochondrial protein expression, induce mitochondrial synthesis, and enable mitochondrial function to play an effective role[7]. Importantly, the role of PGC-1α in neurons has also been demonstrated. It has been reported that the increased expression of PGC-1α has a protective effect in neurons[8]. At the same time, our previous studies also found that the over-expression of PGC-1α in transgenic mice decreased the neural defect and infarct size induced by ischemia-reperfusion (I/R) injury, and the expression levels of inflammatory protein and tumor necrosis factor were also significantly decreased. And nuclear factor erythroid-2 related factor 2 (Nrf2), heme oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1) protein expression increased[9]. These results suggest that regulating the activity of PGC-1α can promote mitochondrial biogenesis in the process of brain tissue injury, and promote the expression of Nrf2 protein to play an anti-oxidative stress role and inhibit the accumulation of ROS, thus alleviating the damage of hippocampal neurons.

The efficacy of electroacupuncture (EA) in the treatment of cognitive impairment after stroke has been demonstrated[10]. EA can significantly improve neurological deficits in VD patients[11]. However, as an invasive technique, EA therapy may cause needlesickness, needle stagnation, bleeding infection, which may affect the acceptance and compliance of patients[12]. Transcutaneous electrical acupoint stimulation (TEAS) is a treatment technique combining the transcutaneous electrical nerve stimulation with the traditional meridians and acupoints theory[13]. It mainly delivers electric current through electrodes attached to acupoints. It has the advantages of non-invasive, painless, convenient operation and no effect on patient level[14]. In our early clinical study, TEAS was used to stimulate the Shenting, Baihui and other acupoints of patients with cognitive dysfunction, and it was found that the patients' daily activity ability and cognitive function were improved, but the mechanism of action still needs further study[15]. The purpose of this study was to explore the effects of TEAS on cognitive function and the mechanism of VD rats. And expect to provide a necessary experimental basis for early application of TEAS in the treatment of VD patients with chronic cerebral

hypoperfusion.

1 Materials and methods

1.1 Animals

A total of 36 healthy male SD rats weighing 220–250 g were obtained from Hunan Lake Jingda Experimental Animal Co., Ltd. All rats were raised in the Experimental Animal Center of Gannan Medical University under the following conditions: a consistent temperature (22±2)℃; relative humidity for 50%–60%, alternating lighting for 12 h. They were adaptively fed for 7 d and provided with adequate food and water. All experimental protocols were approved by the Biomedical Research Ethics Committee of Gannan Medical University (2021013).

1.2 Experimental groups and VD model establishment

Rats were randomly divided into four groups: Sham group, Model group, TEAS group and EA group, with 9 rats in each group. Rats in Model group, TEAS group and EA group were modeled by modified 2-VO. Before modeling, rats were fasted for 12 h but drank freely. After anesthesia with 10% chloral hydrate intraperitoneal injection (3 ml/kg), the rat was fixed in a supine position on the surgical plate. After disinfection, the left CCA, external carotid artery (ECA) and ICA were separated along the midline neck incision of rat. The left ICA was ligated permanently with sutures, and the rat was placed on a heating pad after suturing skin. After recovery, all rats were put back into cage in groups and given a small amount of normal saline to supplement body fluids. One week later, ICA was ligated on the right side of the rat under the same conditions. In Sham group, rats underwent same procedure, except for the ICA ligation.

1.3 Therapeutic method

According to the reference[12], TEAS acted on Baihui and Zusanli points. Treatment parameters of TEAS: frequency 15 Hz, dense wave, current intensity 20 mA (cause rats to nod like action and lower limb muscle contraction), 30 min/time, once a day, continuous stimulation for 14 d. EA also acted on Baihui and Zusanli. Treatment parameters of EA: frequency 1–2 Hz, dense wave, current intensity 1.5 mA, 30 min/time, once a day, continuous stimulation for 14 d. Rats in Sham and Model groups were fixed in the same way every day without any intervention.

1.4 Behavioral evaluation

1.4.1 Novel object recognition test

The experimental equipment includes an open-field box, a camera, and objects A1 and A2 (cubes) of the same size and object B (cylinders). One day before the formal test, the rats were put into open-field box without any objects for 5 min to adapt to the experimental environment. During the formal test, A1 and A2 were presented in two fixed positions on one side of the open-field box, and the rats were placed in the middle of the other side of the open-field box with their back to the two objects and then explored freely for 3 min. The time of exploring A1 and A2 was recorded. Then, A1 or A2 was replaced with B, and the rats were also placed in the open-field box for 3 min, and the time of exploring A1 or A2 and B was recorded. The exploration time for A1 or A2 was denoted by “F”, and the exploration time for B was denoted by “N”. The discrimination index (DI) =N/(N+F) was calculated as an indicator to evaluate the learning and memory ability of rats.

1.4.2 Morris water maze test

Morris water maze test is a classic test to evaluate the spatial acquired function in rats. The experimental equipment consists of a round pool and a camera. The pool was divided into 4 quadrants of equal size, with a circular platform of adjustable height placed in the 3rd quadrant. Before each trial, place opaque water in the pool until the platform was submerged 2 cm below the water. During the training phase, the rats were placed in the water from each of the 4 quadrants of the pool in each trial. The time of finding the platform was recorded, which was defined as escape latency. Once the platform was found, rats were allowed to stay on it for 20 s. If rats fail to find the platform within 60 s, they were guided to the platform and stayed for 20 s, and the escape latency was recorded as 60 s. In the formal experiment phase, the platform was removed, and rats were placed in the water from the 1st quadrant. The times of rats crossing the original platform area and the residence time in the original platform quadrant were recorded within 60 s.

1.4.3 Y maze test

This maze is an enclosed apparatus in the form of the letter “Y” with three arms placed horizontally (40 cm×15 cm×35 cm, each angle for 120º). In order

to prevent interference from external light source, the experimental equipment was covered with cloth curtain. The principle of alternation is based on the fact that, the rats prefer to visit less recently visited arm thus implicating that it needs to recall the last arm visited. At the beginning of each trial, rats were placed at the starting point of one arm, and the sequence of entering each arm and the total number of entering arm were recorded within 5 min. Alternation was defined as the rats entered three arms successively. Maximum alternation was defined as the total number of arm entry times of rats minus 2, and calculated the percentage of alternation=alternation/maximum alternation×100%.

1.5 Morphological observation of neurons and mitochondria in hippocampus of rats

1.5.1 Determination of oxidation-related biochemical indicators

To observe the hippocampal neuron structure of rats, Hematoxylin and eosin (H&E) staining was adopted. Paraffin-embedded hippocampus tissues were cut into 4 μm coronal sections, following deparaffinization in xylene and rehydration with graded ethanol. Finally, these tissue sections were stained with H&E staining kit (Solarbio, Beijing, China) according to the manufacturers' instructions. Results were observed under the optical microscope (10×10 fold, 40×10 fold, ERc5S, Carl Zeiss Company).

1.5.2 Transmission electron microscopy (TEM)

The hippocampus samples in 2.5% glutaraldehyde were cut into small pieces and placed in PBS buffer for 3 h, then fixed in 1% osmic acid solution for 2 h. The samples were dehydrated in a graded series of ethanol, embedded with pure epoxy resin, sliced into ultra-thin sections, and then stained with lead and uranium. The mitochondrial ultrastructure of hippocampus were observed by TEM and the images were recorded by a camera.

1.6 Western blot

One part of the samples were centrifuged to extract the supernatant of the total tissue proteins and the other part to extract cytoplasmic proteins and nuclear proteins. The concentration of proteins were determined using a bicinchoninic acid (BCA) kit. 240 μl protein samples were added to 60 μl 5×loading buffer, boiled at 100℃ for 5 min. 2 μl marker was added to the first hole, and 10 μl of denatured protein was loaded into the other each hole, and then separated by separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Samples were then transferred to NC membranes, the membranes were blocked with 1×PBST containing 5% skim milk at room temperature for 90 min, overnight at 4℃ . After incubating at room temperature for another 30 min the next day, the primary antibodies of this study (anti-β-actin (1∶5 000, proteintech, 66009-1-Ig), anti-PCNA (1∶1 000, proteintech, 10205-2-AP), anti-PGC-1α (1∶1 000, abcam, ab54481), anti-Keap1 (1∶2 000, proteintech, 60027-1-Ig), anti-Nrf2 (1∶1 000, Cell Signaling Technology, #12721), anti-HO-1 (1∶1 000, proteintech, 27282-1-AP), anti-NQO1 (1∶10 000, abcam, ab80588), anti-NRF1 (1∶2 000, abcam, ab175932), anti-TFAM (1∶3 000, proteintech, 22586-1-AP)) were diluted in proportion 1×PBST, and the membranes were co-incubated with the primary antibodies for 90 min at room temperature, and then washed with 1×PBST for 3 times, 15 min each. Next, the secondary antibodies (HRP goat anti-mouse IgG (1∶5 000, proteintech, SA00001-1), HRP goat anti-rabbit IgG (1∶6 000, proteintech, SA00001-2)) were diluted with 1×PBST in proportion, and the membranes were co-incubated with the secondary antibodies for 90 min at room temperature, and then washed with 1×PBST for 3 times, 10 min each. The films were developed with enhanced chemiluminescence (ECL) developer for 1 min, then dried by the filter papers. The hybrid films were wrapped with plastic film, and then exposed with CL-Xposure™ films in a dark box. Quantitative analysis of the densities of protein bands was performed using a quantity one professional grayscale analysis software.

1.7 Statistical analysis

Statistical analysis was performed with SPSS 23.0 software. All data were presented as the mean± SEM. When the data met normal distribution and homogeneity of variance, one-way ANOVA followed by an appropriate post-hoc test was used to test the differences among multiple groups. When variance was not uniform, Dunnett's T3 post-hoc test method is used to correct. When data were not normally distributed, the Kruskal-Wallis test followed by an appropriate post-hoc test was used to test the differences among multiple groups, and the Mann-Whitney U test was used was used to compare the

differences between two groups. $P<0.05$ was considered statistically significant.

2 Results

2.1 TEAS improves the spatial memory and learning deficits in VD rat

As shown in Figure 1a, the discrimination index of Model group was lower than that of Sham group ($P<0.001$). After treatment, the discrimination index of TEAS group and EA group was higher than that of Model ($P<0.01$, $P<0.05$). The results suggested that after TEAS treatment, the memory of familiar and novel objects can be improved in VD rats.

As shown Figure 1b−d, compared with Sham group, the escape latency of rats in Model group was increased ($P<0.001$), the times of crossing the original platform area and the residence time in the original platform quadrant were decreased ($P<0.001$). After treatment, the escape latency of TEAS group and EA group was decreased ($P<0.001$, $P<0.001$), the times of crossing the original platform area ($P<0.01$, $P<0.01$) and the residence time in the original platform quadrant were increased ($P<0.05$, $P<0.01$). The results indicated that spatial memory of VD rats was improved after TEAS treatment, and the effect is better than EA.

As shown in Figure 1e, the percentage of alternation in Model was lower than that in Sham ($P<0.001$). After treatment, the percentage of alternation between TEAS and EA groups increased compared with Model group ($P<0.05$, $P<0.05$). The results showed that the spatial working memory of VD rats were improved after TEAS treatment.

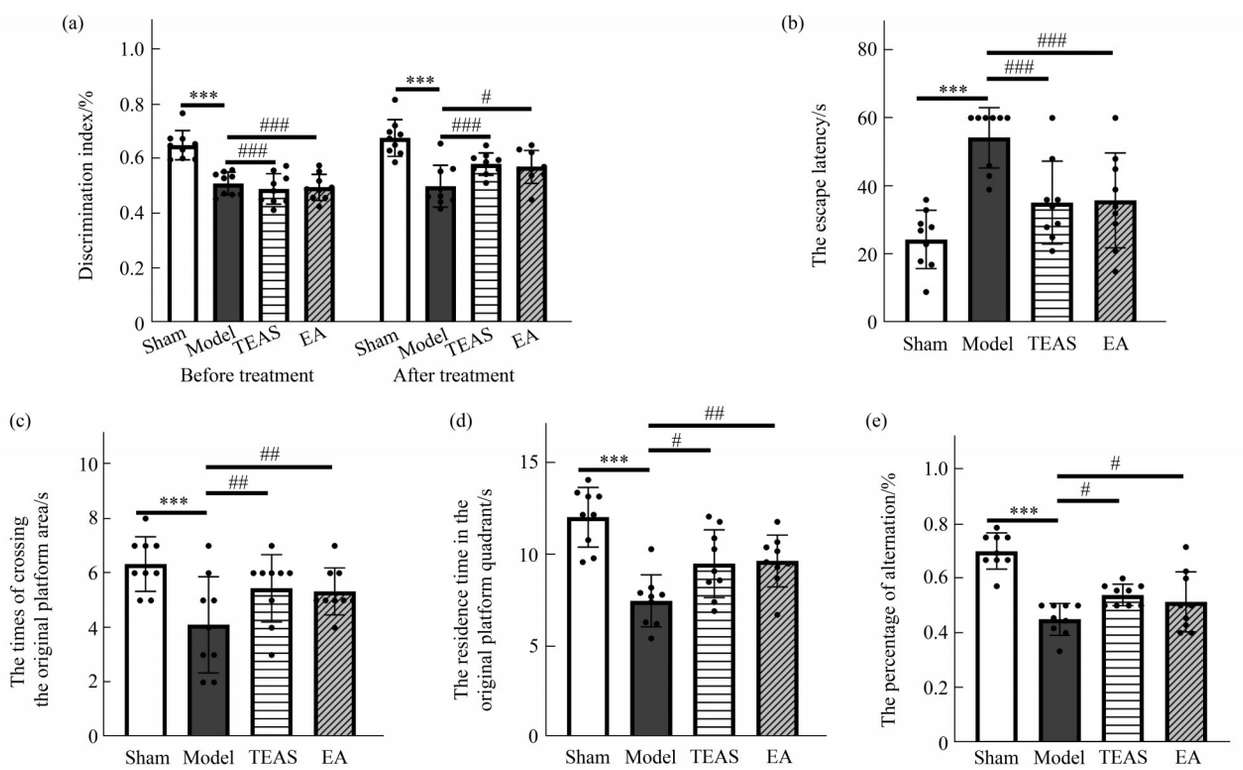

Fig. 1 The results of neurobehavioral tests

(a) The rats' discrimination index of novel object recognition test. (b) The rats' escape latency of Morris water maze test. (c) The rats' times of crossing the original platform area of Morris water maze test. (d) The rats' residence time in the original platform quadrant of Morris water maze test. (e) The rats' alternation percentage of Y maze test. ***$P<0.001$, compared with Sham group; #$P<0.05$, ##$P<0.01$, ###$P<0.001$, compared with Model grou.

2.2 TEAS reduces hippocampus neuron injury in VD rat

As shown in Figure 2, hippocampal neurons in Model group were disordered, the gap were enlarged, the morphology of some neurons were irregular, the boundary of nuclear membrane and nucleoli were unclear, and the nucleuses were pyknotic or dissolved.

After 14 d of treatment, the morphology of hippocampal neurons in TEAS and EA groups were more regular and evenly distributed, with clear nuclear membrane and nucleoli and fewer necrotic neurons. The results suggested that hippocampal neuron damage was increased in Model group, and hippocampal neuron damage was improved in TEAS group and EA group.

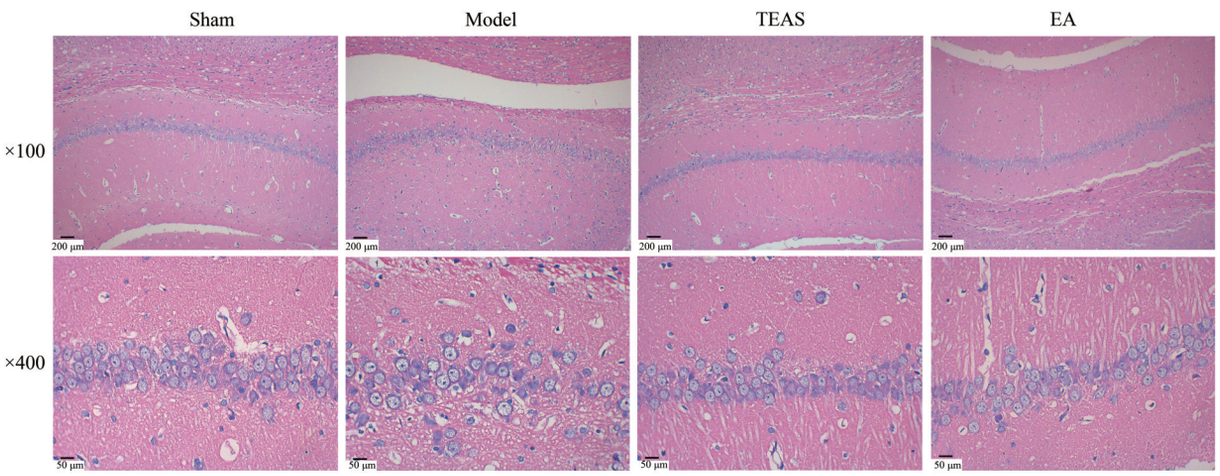

Fig. 2 The pathomorphology of neurons in hippocampus by H&E staining

2.3 TEAS improves the ultrastructure of hippocampal mitochondria in VD rat

As shown in Figure 3, mitochondria of hippocampal neurons in Model group showed swelling, and disappearance of cristae dissolution. Compared with Model group, the mitochondrial ultrastructural pathological changes in TEAS and EA groups were reduced, showing that mitochondrial swelling were reduced, matrix density were increased, and mitochondrial cristae were more obvious.

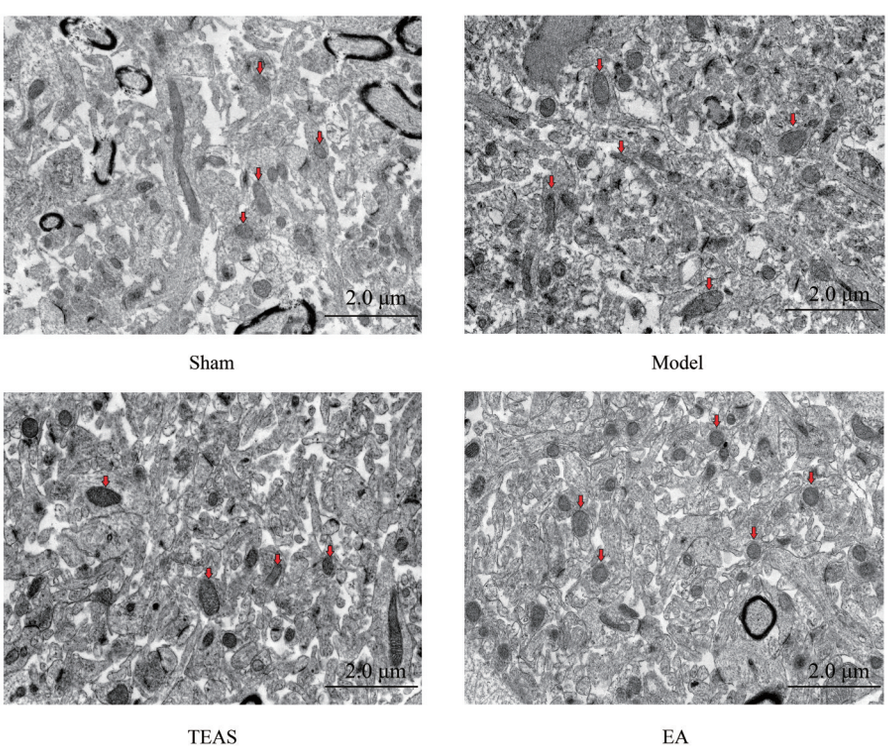

Fig. 3 The mitochondrial subcellular structure in hippocampus by TEM

Mitochondria are labelled with red arrows.

2.4 TEAS suppresses oxidative stress in serum of VD rat

As shown in Figure 4, compared with Model group, the levels of SOD (P<0.001, P<0.01), GSH-Px (P<0.001, P<0.01) and CAT (P<0.001, P<0.001) in rats' serum of TEAS and EA groups were significantly increased, the concentration of MDA (P<0.001, P<0.01) and the content of ROS content decreased (P<0.001, P<0.001). Compared with EA group, the levels of SOD, GSH-Px and CAT in rats' serum of TEAS group were increased and the content of MDA and ROS were decreased.

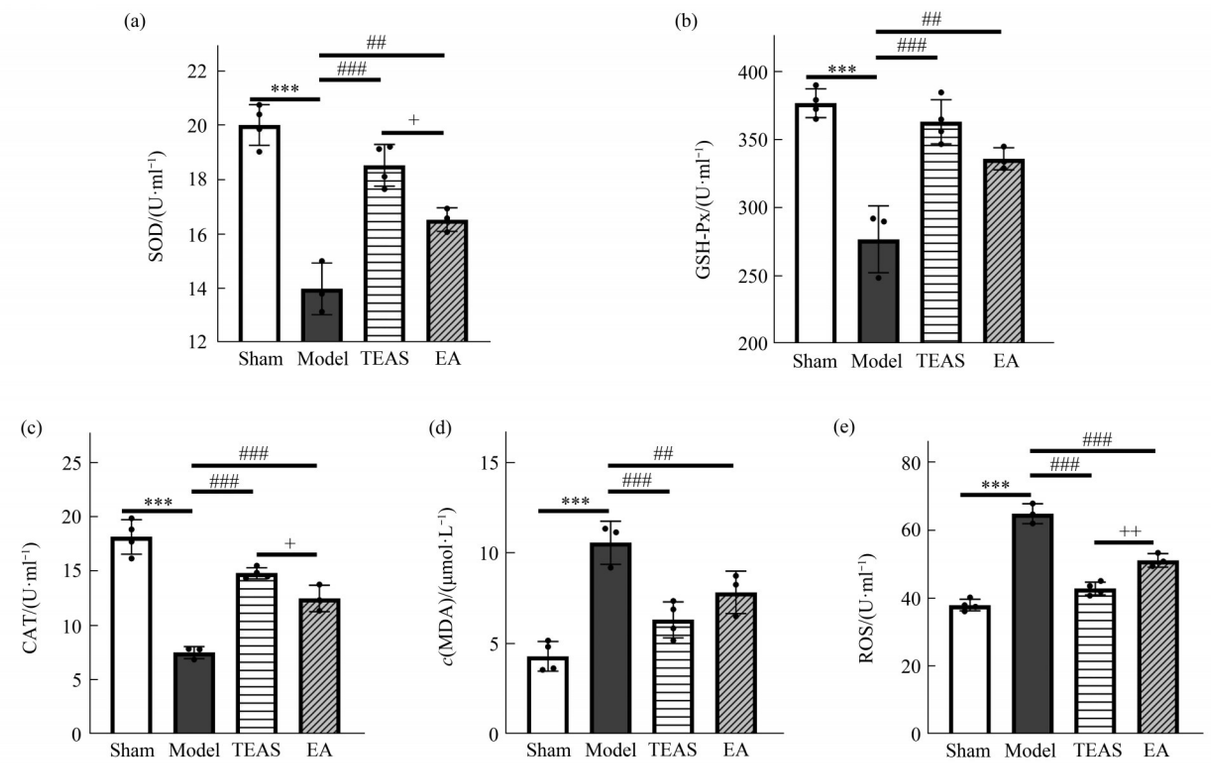

Fig. 4 The indexes of oxidative stress in the serum

(a) The value of SOD activity. (b) The level of GSH-Px. (c) The level of CAT. (d) The concentration of MDA. (e) The content of ROS. ***P<0.001, compared with Sham group; ##P<0.01, ###P<0.001, compared with Model group; +P<0.05,++P<0.01, compared with TEAS group.

2.5 TEAS increases the expression of PGC−1α, NRF1, TFAM proteins in the hippocampus of VD rat

As shown in Figure 5, compared with Sham group, the expression of PGC-1α and TFAM proteins in the hippocampus and NRF1 protein in nucleus of Model group were decreased (P<0.001); compared with Model group, the expressions of PGC-1α (P<0.001, P<0.001) and NRF1 (P<0.001, P<0.05) and TFAM (P<0.001, P<0.01) proteins of TEAS and EA groups were increased.

2.6 TEAS increases the expression of Nrf2, NQO−1 and HO−1 proteins in the hippocampus of VD rat

As shown in Figure 6a, b, compared with Sham group, the expression of Nrf2 protein in the nucleus of hippocampus of Model group were decreased (P<0.001); compared with Model group, the expression of Nrf2 protein in the nucleus of hippocampus were increased in TEAS and EA groups (P<0.001, P<0.05).

As shown in Figure 6c−e, the expression of HO-1 and NQO1 proteins in hippocampus of Model group were decreased compared with Sham group (P<0.001, P<0.001); compared with Model group, the expression of HO-1 (P<0.05, P<0.05) and NQO1 (P<0.01, P<0.01) proteins in hippocampus of TEAS and EA groups were increased.

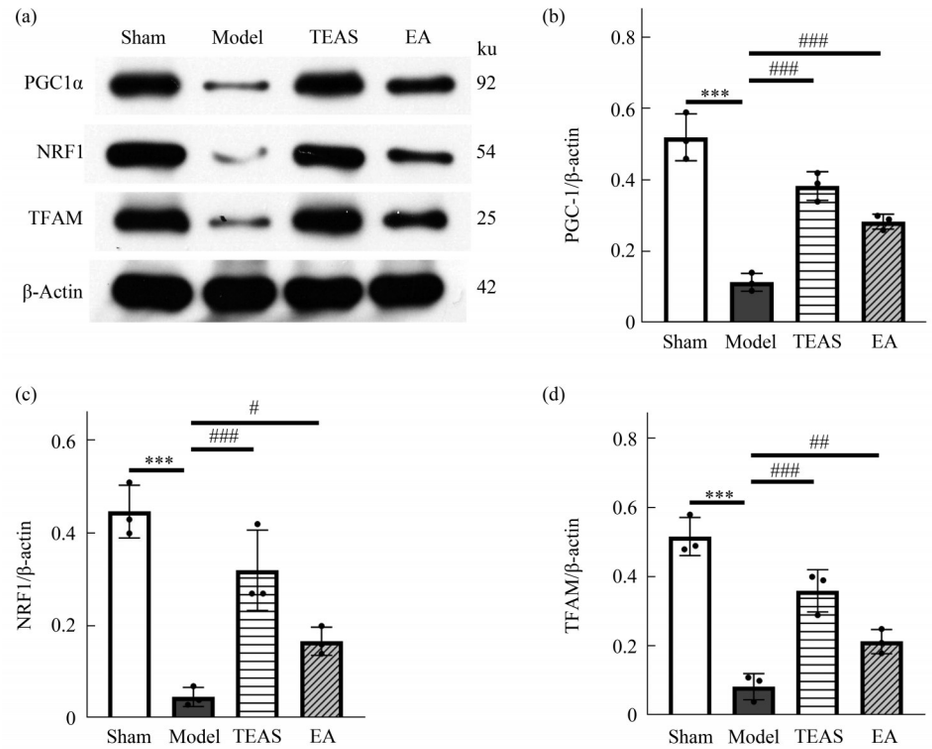

Fig. 5　The protein expression of PGC−1α, NRF1 and TFAM in hippocampus

(a) The electrophoresis images of PGC-1α, NRF1 and TFAM proteins. (b) The quantitative analysis of PGC-1α protein. (c) The quantitative analysis of NRF1 protein. (d) The quantitative analysis of TFAM protein. ***P<0.001, compared with Sham group; #P<0.05, ##P<0.01, ###P<0.001, compared with Model.

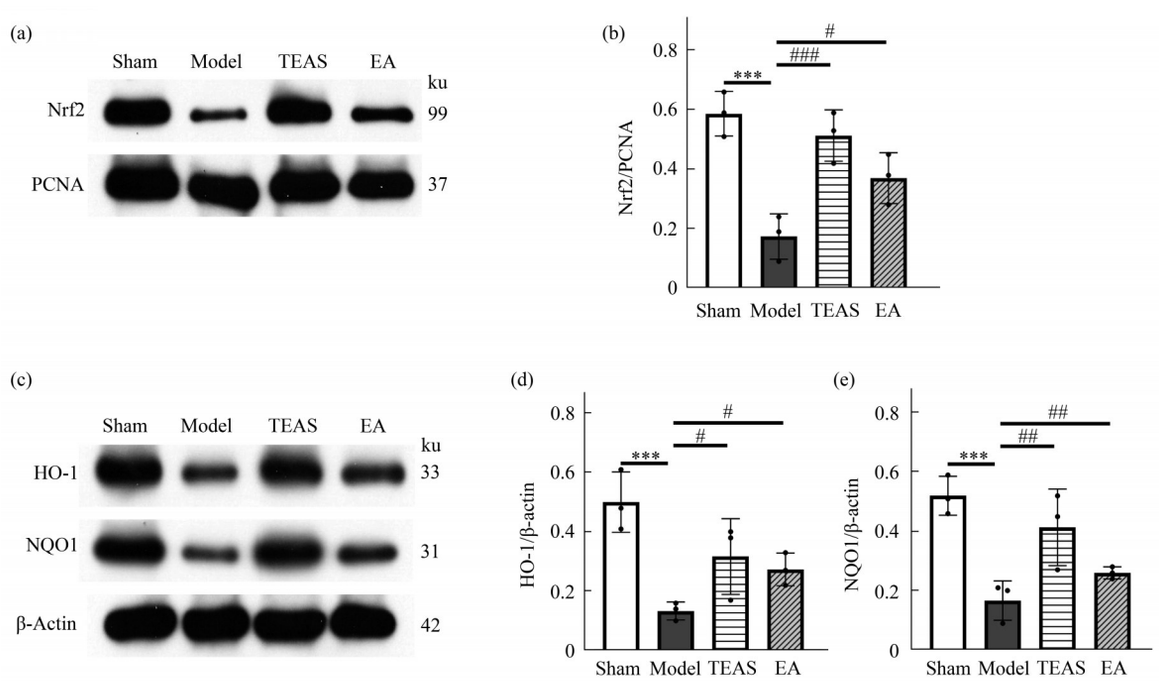

Fig. 6　The protein expression of Nrf2, HO−1 and NQO1 in hippocampus

(a) The electrophoresis images of Nrf2 and PCNA proteins. (b) The quantitative analysis of Nrf2 protein. (c) The electrophoresis images of HO-1 and NQO1 proteins. (d) The quantitative analysis of HO-1 protein. (e) The quantitative analysis of NQO1 protein. ***P<0.001, compared with Sham group; #P<0.05, ##P<0.01, ###P<0.001, compared with Model group.

3 Discussion

TEAS was used to stimulate the Baihui and Zusanli points of VD rats for 14 d and observed its effects on the cognitive function of VD rats. The results suggested that TEAS can improve the learning and memory function of VD rats, and the effect is better than EA.

The principle of low-frequency electrical stimulation in the treatment of nervous system diseases is to enhance the excitability of the brain through the sensory impulse generated by electrical stimulation to the cerebral stem reticular structure, and then through the ascending reticular excitatory system in the ascending non-specific system, so as to promote the activation of neurons and signal transduction[13]. TEAS have been shown to be an effective low-frequency electrical stimulation method of relieving pain[14]. Recent clinical studies have shown that TEAS can improve post-operative cognitive impairment[15]. In addition, our previous clinical studies showed that TEAS can improve cognitive impairment after stroke, but the exact mechanism is unclear[16]. EA and TEAS are clinical rehabilitation treatment methods that applies electrical current to specific acupoints, and numerous studies showed EA can improve cognitive impairment caused by a variety of diseases, including AD, vascular dementia, and postoperative cognitive impairment[17]. TEAS is similar to EA in principle, except the way in which an electric current is delivered. On the basis of clinical experiments, we selected representative Baihui and Zusanli points to explore the effects of TEAS on cognitive function of VD rats and the mechanism, and compared with EA. In our study, TEAS and EA treatments improved the rats' ability to recognize new and old objects as well as spatial learning and memory. The main performance was that the identification index, the times of crossing the platform, the time of staying in the target quadrant and alternating percentage increased, the escape latency decreased. Our findings indicate that TEAS can enhance the cognitive ability of VD rats, and the therapeutic effect of TEAS is better than that of EA. The reason may be that the frequency and current intensity of TEAS is different from that of EA, which can better promote the release of neurotransmitters and the activation of neurons. As previously stated, TEAS is a painless and non-invasive treatment method, whereas EA is an invasive technology that may result in discomfort and impact the effectiveness of the treatment to some degree.

ROS is a byproduct of mitochondrial oxidative phosphorylation. It has a strong catalytic and toxic effect on cells directly, and can also indirectly generate oxygen free radicals that react with DNA, proteins and lipids to induce cell death[18]. Under normal physiological conditions, a variety of endogenous antioxidant enzymes exist in cells, which can avoid ROS accumulation and damage of oxygen free radicals[19]. However, hypoxic-ischemic brain injury leads to loss or mutation of mtDNA in hippocampal neurons vulnerable to damage by oxygen free radicals, resulting in reduced expression of respiratory chain enzyme complex protein subgene[20]. There is a pre-feedback cascade regulation relationship between mtDNA abnormality and ROS production, which leads to the imbalance of oxidation and antioxidant reduction system in brain[21], and endogenous antioxidants are consumed by free radicals, which is manifested by decreased the levels of SOD, GSH-Px and CAT, ROS accumulation and MDA generation. In this study, we found that TEAS and EA increased the levels of SOD, GSH-Px and CAT, decreased the levels of ROS and MDA, and reduced the oxidative stress response in VD rats. Furthermore, in this study, with TEM and biochemical detection, we also found that VD rats hippocampal mitochondria ultrastructure pathological changes and increased the level of oxidative stress *in vivo*, and as after treatment of rats hippocampal mitochondria ultrastructure was improved, the level of oxidative stress decreased *in vivo*.

PGC-1α has been shown to play an important role in both mitochondrial biogenesis and anti-oxidant processes[22]. Some studies have found that the decreased expression of PGC-1α was directly proportional to the number of neuron death, the mechanism may be related to the decreased expression of PGC-1α leading to the decreased level of mitochondrial anti-oxidant protein and uncoupling protein[23]. However, PGC-1α over-expression can significantly up-regulate the expression of mitochondrial anti-oxidant protein and uncoupling protein, inhibit the production of ROS, and ultimately improve the cognitive dysfunction caused by chronic cerebral hypoperfusion in mice. Notably, PGC-1α can

sense the signals of nutrition, environment, and development, and once PGC-1α is activated, it is recruited into the nucleus, where it regulates nuclear respiratory factor 1 (NRF1), promoting the expression of electron transfer chain subunits encoding nuclear genomes. Meanwhile, NRF1 indirectly promotes the expression of mitochondrial transcription factor A (TFAM), which can combine with D-loop region of mtDNA to participate in the replication of mitochondrial genome, and promote mitochondrial biogenesis and metabolic adaptation. Studies have found that most neurodegenerative diseases were accompanied by decreased expression of PGC-1α and NRF1, which further blocked transcription and expression of TFAM, resulting in a sudden decrease in mtDNA quantity, and blocked expression of respiratory chain related proteins, abnormal mitochondrial oxidative phosphorylation, and increased production of ROS[24]. In this study, TEAS and EA activate PGC-1α mediated mitochondrial biogenesis, with increased expression of PGC-1α, NRF1 and TFAM proteins in hippocampus.

In addition, researchers found that increased expression of PGC-1α can also activate Nrf2 to regulate the transcription of anti-oxidant genes, thereby alleviating the damage caused by oxidative stress[25]. Nrf2 is the main regulator of endogenous antioxidant mechanism. Studies showed that the expression of anti-oxidant genes and proteins in *Nrf2* gene knockout mice was significantly reduced, and the oxidative stress was significantly aggravated[26]. Under physiological conditions, Nrf2 and Kelch-like ech-associated protein1 (Keap1) are coupled and stable in the cytoplasm, forming complex and remaining inactivated, and can be degraded by ubiquitination. Oxidative stress can activate the uncoupling of Nrf2 and Keap1, promote Nrf2 to enter the nucleus and bind with Maf small protein to form isodimer, and then bind with antioxidant response elements (ARE), thus initiating the expression of anti-oxidant enzyme factors located downstream, such as HO-1 and NQO1. Subsequently, the endogenous antioxidant responses are activated to increase the resistance of cells to oxidative stress stimulation, thus playing a role in vascular protection. However, the specific signaling pathway between PGC-1α and Nrf2 has not been fully elucidated. In this study, we found that TEAS up-regulated the expression of HO-1 and NQO1 proteins in hippocampus.

In conclusion, this study suggests that TEAS may promote mitochondrial biogenesis by up-regulating the expression of PGC-1α, NRF1 and TFAM proteins in hippocampus, and further activate the expression of antioxidant proteins Nrf2, HO-1 and NQO1, thus increasing the contents of antioxidant enzymes SOD, GSH-Px and CAT in serum, decreased the production of ROS and MDA, and finally improved the cognitive function of VD rats.

4 Conclusion

TEAS can improve cognition, hippocampal neurons and mitochondrial structure of VD rats, and the effect is better than EA. The mechanism may be the activation of PGC-1α mediated mitochondrial biogenesis and antioxidant stress, which also provides a potential therapeutic technology and experimental basis for the treatment of VD.

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经皮穴位电刺激通过PGC-1α介导的线粒体生物生成和抗氧化应激改善血管性痴呆大鼠的认知功能*

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摘要 **目的** 探讨经皮穴位电刺激（transcutaneous electrical acupoint stimulation，TEAS）对血管性痴呆（vascular dementia，VD）大鼠认知功能的影响及其机制。**方法** 采用改良的双血管闭塞（2-VO）法建立VD大鼠模型。造模后分别采用TEAS和电针（EA）刺激大鼠百会穴和足三里穴，连续刺激14 d。治疗后，采用新物体识别实验、Morris水迷宫实验、Y迷宫实验评估大鼠空间记忆和学习能力。苏木精-伊红染色观察海马神经元形态；透射电镜观察海马线粒体超微结构；采用酶联免疫吸附测定试剂盒检测大鼠血清中SOD、CAT、GSH-Px、MDA和ROS水平。采用蛋白质免疫印迹法（Western blot）检测各组大鼠海马组织中PGC-1α、TFAM、HO-1、NQO1蛋白及细胞质中Keap1蛋白及细胞核中Nrf2、NRF1蛋白的表达。**结果** 治疗14 d后，与模型组比较，VD组大鼠逃避潜伏期缩短，辨别指数、穿越原平台区域次数、原平台所在象限停留时间、交替百分比增加；TEAS可改善VD大鼠海马神经元及线粒体结构，病理染色结果显示神经元排列更规则、分布更均匀，核膜、核仁更清晰，线粒体肿胀减轻，线粒体基质密度增加，线粒体嵴更明显。血清中SOD、GSH-Px和CAT水平显著升高，MDA和ROS浓度降低。TEAS还上调了海马区PGC-1α、TFAM、NQO1、HO-1蛋白和核内NRF2、NRF1蛋白的表达水平，但下调了胞浆中Keap1蛋白的表达。**结论** TEAS可改善VD大鼠的认知功能，改善海马神经元和线粒体结构，且效果优于电针，其机制可能是激活PGC-1α介导的线粒体生物发生和抗氧化应激，这也为VD的治疗提供了潜在的治疗技术和实验依据。

关键词 血管性痴呆，经皮穴位电刺激，认知功能，PGC-1α，抗氧化，线粒体
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