

Research Article

The Influence of Acupuncture on the Expression of PDK and A-KGDHC in Vascular Dementia Rats

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Abstract

Vascular Dementia (VD) is a common dementia disease after Alzheimer's disease. It accounts for 20% of dementia patients. Worse still, there is no drug for treating vascular dementia. A large number of studies have shown that chronic cerebral ischemia is an important inducement factor of vascular dementia and Alzheimer's disease. Ischemia inevitably leads to insufficient energy supply in the brain. In this study, we observed the effect of acupuncture on the expression of pyruvate dehydrogenase synthase (PDK) and Alpha Ketoglutaric Dehydrogenase (α -KGDHC) in the brain of 2-VO rats. The experimental results showed that acupuncture could improve learning and memory ability of VD rats, increase the expression of PDK and α -KGDHC and increase the content of ATP in brain. It indicates that acupuncture can improve learning and memory ability by enhancing expression of PDK and α -KGDHC in VD rats.

Keywords: Acupuncture; Vascular dementia; ATP; PDK; α -KGDHC

Introduction

Vascular Dementia (VD) is a common dementia disease after Alzheimer's disease. It accounts for 1/5 of the dementia [1]. Similar to Japan, VD has a higher proportion in China than Alzheimer's disease [2]. However, the pathogenesis of vascular dementia is still unclear. Cerebral blood vessels lesions, caused by vascular risk factors, play an essential role in the development of vascular dementia. Numerous researches show that reduced global cerebral ischemia and reperfusion can lead to brain dysfunction and cognitive decline [3-5]. These conditions can obviously cause a shortage of brain energy supply. And some metabolic syndromes like insulin resistance and dyslipidemia are associated with lower cognitive performance [6]. The insulin resistance is relate to lower arterial blood flow and reduced cortical perfusion [7]. Insulin signal disorder can cause the reduction of energy substrate, such as ATP in the brain. Recent reports have shown that improving the metabolism of ATP in the brain may be a potential way to treat cerebral ischemic diseases [8-10].

As we all know, the tricarboxylic acid cycle (TCA) is the main physiological process of ATP formation. When the body is short of oxygen supply, the activity of this process weakens, resulting in the decrease of ATP production, which hinders the normal function of brain [11]. Pyruvate dehydrogenase synthase (PDK) and Alpha Ketoglutaric Dehydrogenase (α -KGDHC) are two key enzymes of aerobic oxidation of glucose. Under aerobic conditions, pyruvic acid enters TCA cycle through the action of PDH. While α -KGDHC is the key step to control the cycle smoothly. A recent research shows that regulation of PDK1 can control the development of some risk factors of vascular dementia such as hypertension, obesity, diabetes and stroke [12].

In Traditional Chinese Medicine (TCM), there is no title of vascular dementia. But it can be divided into dementia, forgetfulness and madness disorder based on its clinical symptoms. Acupuncture

has been used for more than 2000 years in TCM. Its main role in TCM is to dredge the main and collateral channels, regulate the Yin and Yang and strengthening the body resistance to eliminate pathogenic factors. Acupuncture can be used in the treatment of facial nerve paralysis [13], trigeminal neuralgia [14,15], hemiplegic and other systemic diseases [16]. Increasing researches have confirmed that acupuncture therapy has a definite improvement in VD [17-19]. In this study, we acupuncture 2-VO model rats Baihui and Shen men points. It is found that it can promote the expression of PDK and α -KGDHC in brain tissue, improve ATP level, and improve learning and memory ability.

Materials and Methods

Animals

25 male and 25 female Sprague-Dawley (SD) rats (240 \pm 20g) were purchased from Institute of laboratory animals of Sichuan Academy of Medical Sciences (Chengdu, China). All animals were fed freely and housed under suitable temperature (22 \pm 2 $^{\circ}$ C) and humidity (65 \pm 5%) conditions in the animal observation room. And they were treated with ethical practices adhering to international regulations (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996).

Model preparation

All rats fed a week later for preparing 2-VO model [20]. Briefly from the middle of the neck after they were anaesthetized by pentobarbital sodium solution. The bilateral common carotid arteries were separated and ligated with 5-0 silk threads. The sham operation group underwent the same operation procedure without ligating the common carotid artery. Until the anesthesia was revived, the body temperature of the rats was maintained at 37.5 $^{\circ}$ C by electric blanket. After that, the rats were housed in cages with free diet. After 15 days of recovery and quarantine, they were used for subsequent experimental process.

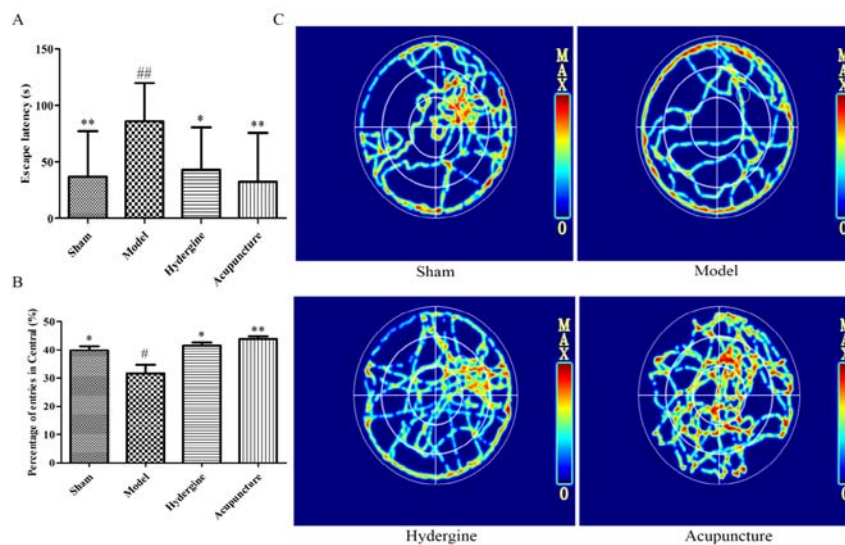


Figure 1: Effect of acupuncture on learning and memory in VD rats. Compared to the sham group, *P<0.05, **P<0.01; compared to the model group, #P<0.05, ##P<0.01.

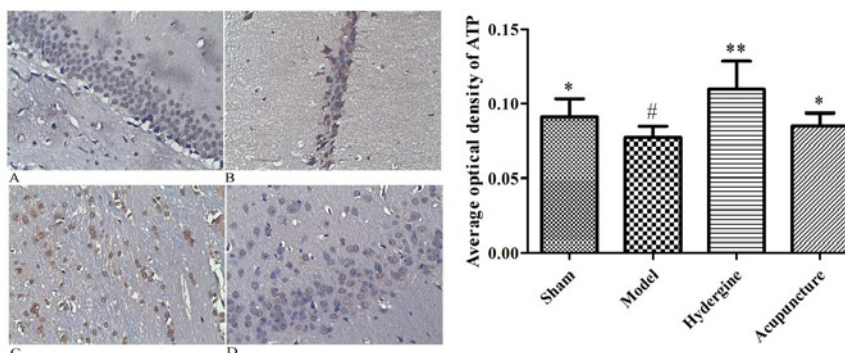


Figure 2: Effect of acupuncture on the expression of ATP in brain tissue in VD rats. Compared to sham group, *P<0.05, **P<0.01; compared to model group, #P<0.05, ##P<0.01. A. the sham group; B. the model group; C. the Hydergine group; D. the acupuncture group.

Groups and treatment

Two weeks after modeling, 30 rats were selected (male and female each half). And then they were randomly divided into model group, hydergine group (0.7mg/kg), acupuncture group according to their weight. Another 10 sham operated rats were taken as the control group (male and female each half). The rats of hydergine group were treated with hydergine (Tianjing Huajin Pharmaceutical Co., Ltd, Tianjing, China), the other three groups were treated with distilled water (10ml/kg). The rats in acupuncture group were prodded at *Baihui* and *Shenmen* according to *Experimental Acupuncture*. The needles were horizontally stabbed into *Baihui* to the front of the rats (0.5 inches) and vertically into *Shenmen* (0.1inches). Rotating the needles 3 minutes and retaining them with G6805 electric needle instrument (Qingdao Xincheng Industrial Co., Ltd., Qingdao, China) for 20 minutes. 1 times a day was treated with acupuncture, and 10 d was 1 courses. After the 1 course of treatment, the rats were resting for 2 day and then needled. After 3 courses of acupuncture, the acupuncture treatment ended. The rats in the other groups also received the same fixed operation as the acupuncture group.

Morris water maze

All rats underwent the Morris water maze test, which evaluates spatial learning and memory performance. Each rat was trained to locate the hidden platform on each of five consecutive days. The animals were given 120s to search the platform. If the rat failed to reach the platform within 120s, it was guided to the location and placed on the platform for 10s before removed from the pool. Twenty-four hours after the last training session, the hidden platform was removed and the rats were permitted to swim freely for 120s. All date was collected and analyze using a charge-coupled device camera placed directly over the pool and coupled with tracking program (Chengdu Tech man Software Co Ltd., Chengdu, China). All tests were performed after 6:00 pm.

Immunohisto chemistry

After the Morris water maze test, the rats were executed by cervical dislocated. Separation of Brain tissue on the ice and fixed with 4% polyformaldehyde. The expression of ATP, PDK and A-KGDHC protein was detected by immunohistochemical (SP). In brief, 5% BSA solution blocked for 20 minutes at room temperature, and then

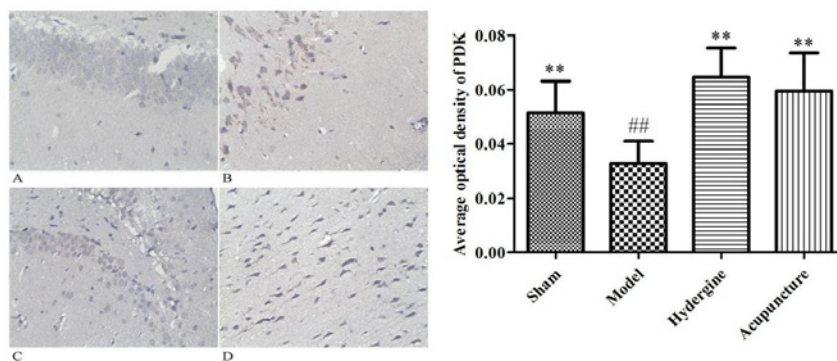


Figure 3: Effect of acupuncture on the expression of PDK in brain tissue in VD rats. Compared to sham group, [#]P<0.05, ^{##}P<0.01; compared to model group, ^{*}P<0.05, ^{**}P<0.01. A. the sham group; B. the model group; C. the Hydergine group; D. the acupuncture group.

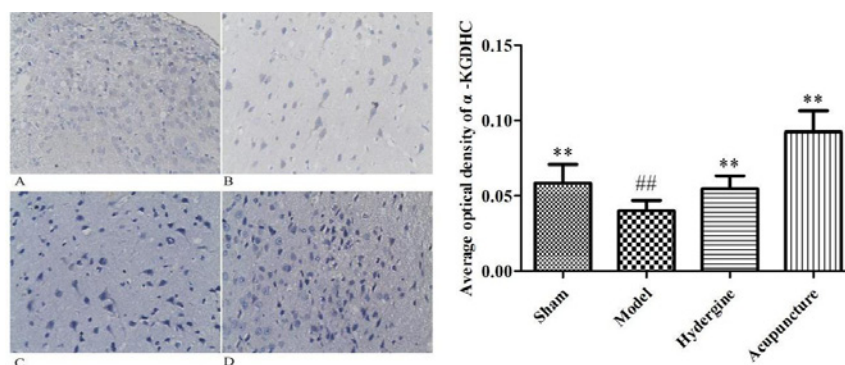


Figure 4: Effect of acupuncture on the expression of alpha-KGDHC in brain tissue in VD rats. Compared to sham group, [#]P<0.05, ^{##}P<0.01; compared to model group, ^{*}P<0.05, ^{**}P<0.01. A. the sham group; B. the model group; C. the Hydergine group; D. the acupuncture group.

discard the excess liquid. Anti-ATP (1: 200, Santa Cruz, USA), anti-PDK (1:250, Santa Cruz, USA) and anti-alpha-KGDHC (1:200, Santa Cruz, USA) were incubated overnight at 4°C. After cleaning, biotinylated goats to mice/rabbit IgG (Beijing Zhongshan Jinqiao biological Co., Ltd., China) was added and incubated for 30 minutes at 37°C. Then S-V/HRP (Beijing Zhongshan Jinqiao biological Co., Ltd., China) was added and incubated for 30 minutes at 37°C, followed by routine operation. The micro camera system of BA200 Digital digital three mesh camera system (Motic China Motic, Xiamen, China) was used to collect images. Each slice was observed at 100 times of the whole tissue. And 1 region was selected to collect the images according to the size and expression of the tissue. The Image-Pro plus 6 image analysis system (Media Cybernetics, Rockville, USA) is used to measure the average optical density of all the images collected. If each slice is taken for multiple images, the average value is obtained.

Statistical analysis

All data are shown as the mean ± Standard Deviation (SD). Each group of data was analyzed by Anderson-Darling. Samples with normal distribution were analyzed by one-way ANOVA. If the sample variance was not homogeneous with Fisher’s Least Significant Difference (LSD) test, the Kruskal-Wallis (K-W) test was used. Non-parametric tests were used for data that was not normally distributed. P<0.05 was regarded as statistically significant.

Results

Effect of acupuncture on learning and memory in VD rats

As shown in (Figure 1A), compared to the sham group, the escape latency of the model group was significantly longer than that of the sham group (P<0.01). The rats in the model group were mainly moved in the outer ring but performed less in the middle ring (Figure 1C). The percentage of entering the middle ring was obviously lower than that of the sham group (P<0.05) (Figure 1B). Compared to model group, the escape latency of the acupuncture group was significantly shortened (P<0.01) (Figure 1A). The rats accepted acupuncture main activated in the platform quadrant, middle ring and inner ring (Figure 1C). Moreover, the percentage of entering the middle ring was obviously lower than that of model group (P<0.05) (Figure 1B).

Effect of acupuncture on the expression of ATP in brain tissue in VD rats

As shown in (Figure 2), compared to sham group, the expression of ATP in brain tissue of model rat was decreased. Its mean optical density is significantly different from that of the sham group (P<0.05). Compared to model group, the expression of ATP in brain tissue of acupuncture treatment group was increased. Its mean optical density was elevated obviously (P<0.05).

Effect of acupuncture on the expression of PDK in brain tissue in VD rats

As shown in (Figure 3), compared to sham group, the expression of PDK in brain tissue of model rat was decreased. Its mean optical density is significantly different from that of the sham group ($P < 0.01$). Compared to model group, the expression of PDK in brain tissue of acupuncture treatment group was increased. Its mean optical density was elevated obviously ($P < 0.01$).

Effect of acupuncture on the expression of α -KGDHC in brain tissue in VD rats

As shown in (Figure 4), compared to sham group, the expression of α -KGDHC in brain tissue of model rat was decreased. Its mean optical density is significantly different from that of the sham group ($P < 0.01$). Compared to model group, the expression of α -KGDHC in brain tissue of acupuncture treatment group was increased. Its mean optical density was elevated obviously ($P < 0.01$).

Discussion

VD is the only preventable dementia [21]. Modern clinical studies and animal experiments have proved that acupuncture therapy can effectively improve the cognitive function and quality of life of patients with vascular dementia [15,17-19]. In this study, we also observed that acupuncture could improve learning and memory ability in model rats. Further studies show that acupuncture can improve the level of ATP in brain tissue by promoting the protein expression of PDK and α -KGDHC.

The choice of meridians and outpoints is the key to acupuncture. According to a large number of literatures, acupuncture treatment of vascular dementia mainly involves 14 meridians and 73 acupuncture points. *Du meridian*, *gallbladder meridian*, *stomach meridian* and *spleen meridian* are commonly used meridians. *Baihui*, *Sishencong*, *Shenting*, *Fengchi* and *Shenmen* are commonly used acupoints [22]. According to the basic theory of TCM, *Du Meridian* controls the Qi of the whole body. Inadequacy of blood supply and slow blood flow are associated with Qi. The heart is the power organ that supplies the whole blood. While the *heart meridian* is a specific meridian to regulate the cardiac function. The important points of *Baihui* and *Shenmen* respectively in *Du meridian* and *heart meridian*. Therefore, we chose *Baihui* and *Shenmen* points as the intervention points in the treatment of VD rats.

Mitochondrion is the main source of ATP production, and its productivity obstacle is closely related to pathological process of VD and aging [23,24]. The ATP enriched in the mammalian brain tissue is the main source of energy in the brain tissue. It involves cell activation, neurotransmitter release and metabolism [25]. Therefore, the activity of ATP is closely related to the function of neuron plasma membrane and energy metabolism in the brain. The mitochondrial function and structure are severely damaged after cerebral ischemia. It will induce mitochondrial swelling and inflammation, inhibit the activity of PDK and α -KGDHC, impede the oxidative phosphorylation process and reduce the production of ATP [26]. PDK and α -KGDHC is multi enzyme complex, they are participate in the most basic glycosylated metabolism of the body, which is the key link in energy metabolism. They are also play an important role in the maintenance of energy supply and the guarantee of sports energy [27]. Abnormal of TCA and

oxidative metabolism in brain mitochondria will not only lead to the reduction of ATP synthesis, but also cause a lot of path physiological reactions.

The model rat prepared by ligaturing bilateral arteria carotis communes is widely used in many kinds of cerebrovascular diseases. It's a commonly VD model for its symptoms associated with insufficient blood supply in the brain. In this study, we confirm that acupuncture can improve learning and memory ability in this model rats. The expression of PDK, α -KGDHC and ATP in the brain of model rats were significantly lower than the sham rats. After treatment with acupuncture at *Baihui* and *Shenmen*, the expression PDK, α -KGDHC and ATP in the brain of model rats were obviously increased. In summary, the learning and memory ability of VD rats could be improved after treatment with acupuncture at *Baihui* and *Shenmen*. And the mechanism may be related to the regulation of TCA in brain and improving ATP production.

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