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Electro-acupuncture up-regulates astrocytic MCT1 expression to improve neurological deficit in middle cerebral artery occlusion rats

Yan Lu [a], Haijun Zhao [a], Yuan Wang [a], Bingbing Han [a], Tong Wang [b], Hong Zhao [c], Kemi Cui [c], Shijun Wang [a,*]

[a] Department of Experimental Acupuncturology, College of Acumox and Tuina, Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250355, China
[b] School of Nursing, Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250355, China
[c] Department of Systems Medicine & Bioengineering, Houston Methodist Research Institute, Weill Medical College of Cornell University, Houston, TX 77030, United States

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ABSTRACT

Aims: Cerebral ischemia is one of the common diseases treated by electro-acupuncture (EA). Although the clinical efficacy has been widely affirmed, the mechanisms of action leading to the health benefits are not understood. In this study, the role of EA in modulating the lactate energy metabolism and lactate transportation was explored on the middle cerebral artery occlusion (MCAO) ischemic rat model.
Main methods: Repeated EA treatments once daily for 7 days were applied to the MCAO rats and neurological function evaluation was performed. Brain tissues were harvested for lactate concentration examination, immunohistochemical staining, Western blot and qRT-PCR analyses for the expressions of lactate transporter (monocarboxylate transporter 1, MCT1) and glial fibrillary acidic protein (GFAP).
Key findings: The animal behavioral tests showed that the 7-day EA treatments significantly promoted the recovery of neurological deficits in the MCAO rats, which correlated with the enhanced lactate energy metabolism in the ischemic brain. In the cortical ischemic area of the MCAO rats, EA treatments led to the activation of astrocytes, and induced a further increase of lactate transporter (monocarboxylate transporter 1, MCT1) expression in astrocytes at both protein and mRNA levels.
Significance: Our results suggest that the EA treatments activated lactate metabolism in the resident astrocytes around the ischemic area and up-regulated the expression of MCT1 in these astrocytes which facilitated the transfer of intracellular lactate to extracellular domain to be utilized by injured neurons to improve the neurological deficit.

1. Background

Electro-acupuncture (EA) is the modern application of traditional Chinese acupuncture combined with electrotherapy. Cerebral ischemia is one of the common diseases treated by EA in East Asian countries, and the clinical efficacy has been widely affirmed. In animal experiments, repeated EA treatments have been demonstrated to accelerate the restoration of function and help heal the cerebral tissue lesion during cerebral ischemia–reperfusion [9]. Disruption of cerebral energy metabolism occurs during ischemia because of glucose and oxygen deprivation. Studies have shown that lactate is the major energy substrate that has been overproduced by astrocytes in the injured brain and plays neuroprotective role [1]. Specifically, lactate is considered as an obligatory energy substrate for surviving neurons during the recovery period after ischemia [15]. EA treatment has been examined to significantly increase the lactate dehydrogenase (LDH) in rat ischemic brain cells [16], as well as increase the plasma level of lactate in senile mice and ischemia patients [14,17]. However, in order to utilize this energy substrate for neurons to survive during the recovery period, changes in the expression of specific lactate transporters on various cell types in the ischemic micro-environment might be necessary.

Monocarboxylate transporters (MCTs) are responsible for up-taking and releasing of energy substrates including lactate [4]. The MCT family has 14 members, 6 of which have been functionally characterized. Of these, only MCT1–MCT4 catalyze proton-coupled transport of lactate. MCT1 is expressed in most human cells, whereas MCT2 is absent in human tissues. MCT3 expression is largely restricted to the retinal pigment epithelium. MCT4 is expressed strongly only in glycolytic tissues (*e.g.* white muscle) that must export large amounts of lactic acid. In this work, we examined the changes in the expression of MCT1 in a rat model of brain ischemia.

In the study, we found that the increased expression of astrocytic MCT1 in the ischemic brain tissue have been involved in the compensatory mechanism of energy metabolism after cerebral ischemia. EA could improve the recovery of neurological function in MCAO rats by stimulating the lactate energy metabolism of activated astrocytes and further up-regulation of MCT1 in the ischemic brain tissue, which plays an essential role in restoration of brain energy metabolism.

* Corresponding author at: 4655 Daxue Road, Changqing District, Jinan, Shandong 250355, China. Tel.: +86 0531 89628505.
E-mail address: feichanglulu@gmail.com (S. Wang).

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2. Materials and methods

2.1. Animals

Healthy male Wistar rats were used for the study (SPF grade, 12 week-aged, body weight 200–220 g), and were purchased from Beijing WeitongLihua Experimental Animal Technology Co., Ltd. The rats were reared in cages where the room temperature was 22 ± 2 °C and the relative humidity was 40%–60%. The experiment began after three day feeding adaptation. All experimental procedures involving rats were approved by the Animal Research and Comparative Medicine Committee of Shandong University of Traditional Chinese Medicine in accordance with the guidelines of IACUC and the regulations.

2.2. Model establishment and experimental grouping

Rats with focal cerebral ischemia model were established by the method of middle cerebral artery occlusion (MCAO) with modifications [10,11]. Thirty-two rats received the MCAO surgery, and were evaluated for their neurological function deficits by a four-point scale (0–4) neurobehavioral test right after awaking from anesthesia (0: no deficit, 1: failure to extend left forepaw fully, 2: circling to the left, 3: falling to the left, 4: no spontaneous walking and loss of consciousness) [10]. Twenty-four out of the 32 MCAO rats with the scores of 2–3 were randomly divided into three groups: model group, EA treatment group and control EA group ($n = 8$, for each group). Eight rats received sham-operation (the same surgical procedures except inserting the nylon monofilament) (sham group), and 8 rats without any operation were served as normal group. The neurological function deficit scale score of the rats in the sham group and normal group were 0.

2.3. EA treatment

Rats in the EA treatment group and control EA group received repeated EA once daily for 7 days. The first EA treatment was right after the neurobehavioral test, within 2 h of finishing the MCAO surgery. Acupuncture was applied with metal needles at 13 mm-length and 0.20 mm-diameter. In the EA treatment group, needles were inserted bilaterally into PC6 (*Neiguan*) in depth of 2 mm and LI11 (*Quchi*) in depth of 4 mm. In the control EA group, needles were obliquely inserted into the muscles between the iliac crest and ribs in depth of 2 mm. Per the "Map of the experimental animal acupoints" formulated by National Acupuncture Research Institute of China, PC 6 is located at 3 mm above the wrist between the radius and ulna on the rat's forelimbs, and LI11 is located at the outside front of elbow near the radius. These two acupoints are commonly used in cerebral ischemia clinic and practically, it's easier to operate on rats than other acupoints on the head or face. The control EA treatment was performed in off-meridian points, which there is no evidence to show their therapeutic effects. The needles were connected with HANS electro-acupuncture therapy apparatus (Jisheng Medical Technology Co., Ltd, Nanjing, China). The following parameters were applied for the treatment: sparse-dense wave (2/15 Hz), intensity at 1 mA, 20 min each session. There was no acupuncture in the normal group, sham group and model group.

2.4. Neurobehavioral tests, tissue collection and sample preparation

Neurological function deficit score for each rat in the EA group was evaluated twice daily (before and 30 min after EA treatment), for the 7 day-treatment duration. Right after the last behavioral test, 5 rats from each group of the 5 groups were anesthetized by intra-peritoneal injection sodium pentobarbital (50 mg/kg), and then perfused with saline (0.9%) via the left ventricular to aorta followed with 4% phosphate-buffered paraformaldehyde. Rat brains were carefully and rapidly removed after perfusion. A 1 cm-thickness coronal brain slice was harvested from the starting point of middle cerebral artery to rostral 0.5 cm and caudal 0.5 cm of the brain. The brain slice was fixed in 4% paraformaldehyde solution, embedded with paraffin, and then continuously sectioned every 5 μm for immunohistochemical staining.

The rest of 3 rats from each group were deeply anesthetized and their brains were rapidly harvested on an ice tray. A 1 cm-thickness coronal brain slice was harvested similarly as described above. The brain slice was grinded in liquid nitrogen for 3 aliquots. One was used for lactate concentration examination, and the other two were used for MCT1 Western blot and qRT-PCR test separately.

2.5. Lactate examination

The lactate concentration was tested by the commercial lactate assay kit (Catalog number: A019-2, Engineering Institute of Nanjing Jiancheng, China) as described before [3].

2.6. Immunohistochemistry

The 2-step plus ploy-HRP Anti-mouse/Rabbit IgG Detection System was used to examine the expression of MCT1 (rabbit anti-MCT1 monoclonal antibody, 1:300, cat# AB3540P, from Chemicon Ltd) and GFAP (mouse anti-GFAP monoclonal antibody, 1:1000, cat# sc-58766, from Santa Cruz Biotechnology) in the 5 μm-thickness brain sections. The microscopic images of the staining were taken under the 10× and 20× objectives of a BX51 microscope (Olympus).

2.7. Western blot

One aliquot brain tissue was thawed and added with RIPA lysate to extract total protein, and then the following procedures were performed including SDS-PAGE gel (12%) electrophoresis, transmembrane, antibody hybridization and exposure imaging. QuantityOne software was used to analyze the strip grey value of the exposure image, with the β-actin as an internal reference.

2.8. Quantitative real-time reverse transcription-PCR (qRT-PCR)

The target gene sequence was obtained from NCBI gene library, using Primer Premier 5.0 (Canadian Premier Life Insurance Company) software to design primers by Beijing xlltech Co. Ltd, China with glyceraldehyde-3-phosphate dehydrogenase (GADPH) as an internal reference. The primer sequences of GFAP are: F-AGAAAACCGCATCACCATTC, R-GCACACCTCACATCACATCC. The primer sequences of MCT1: F-TCGGTATCTTTGGATGGAGAGG, R-TGGTAACTTCATTTGGCTTCCC.

One aliquot brain tissue was used to extract total RNA using the TRIzol kit (Invitrogen). Aliquots of total RNA were reversely transcribed to cDNA using random primers and superscript III reverse transcription kit. cDNA equivalent to 200 ng of total RNA was subjected to the qRT-PCR analysis by the ABI 7500 Fast (Applied Biosystems Company), with the cycling condition as 95 °C for 2 min, and then 94 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s for 40 cycles. The ABI 7500 Fast system software was used to analyze and calculate the expression level of mRNA. The sample from the normal group was used a control. The gene with F value more than 1 is considered upregulated.

2.9. Statistical analysis

All data were analyzed using the SPSS19.0 software (Chicago, IL, USA). All the results were expressed with Mean ± SD and tested for normality. The before and after EA treatment group comparison was analyzed by Paired-Samples T Test. All other two group comparisons were analyzed by In-dependent-Samples T Test (LSD method for homogeneity of variance, and Tamhan's T2 method for heterogeneity of variance). For all statistical tests, a value of $P < 0.05$ was considered statistically significant. All figures were performed using GraphPad Prism 5.0 Software (GraphPad Software Inc., San Diego, CA).