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Research Article

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Effect of electro-acupuncture on the morphology and LRP1 protein expression in the model mice with Alzheimer disease

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ABSTRACT

To observe the influence of electro-acupuncture on the hippocampus morphology and LRP1 protein expression the mice with Alzheimer Disease (AD). Duplicate the AD model mice, SD mice in number 28 were randomly divided into four groups which are control group, model group, treatment group (piracetam 0.62 g•kg-1) and electro-acupuncture group (bilat-eralm Shenshu, Neiguan and Dazhui), 7 mice in each group. The therapy was one time each and it last 28 days. Optical microscopy and electron microscopy were used to observe the morphology, determine the viscera index of the brain and spleen of the mice; enzyme-linked immunosorbent assay (ELISA) was used to determine the Aβ and APP protein levels in the hippocampus; and immunohistochemical technology was used to observe the expression level of the LRP1 protein to determine the effect of electro-acupuncture on the LRP1 signal transduction pathway. Compared with the control group, in the model group mice show the following characteristics: the hippocampus structure was abnormal, the layers of pyramidal cell were reduced, the pyramidal cell were disordered and sparse accompanied with glial cells hyperplasia; the ultra microstructure showed that morphology of the hippocampal neuron was irregular, disordered, nuclear membrane unclear, and mitochondrial swelling, and viscera index of the brain and spleen reduced significantly ($P<0.05$); the level of Aβ and APP proteins increased significantly ($P<0.05$); and the level of LRP1 protein decreased significantly revealed by immunohistochemical detection ($P<0.05$). Compared with the model group, hippocampus structure of the electro-acupuncture group all showed to be restored in different degrees and HE staining showed the neuron arrangement is neat, the structure is compacted; the ultra microstructure showed that morphology of the hippocampal neuron was basically normal, nuclear membrane and mitochondrial are also clearly can be seen, and viscera index of the brain and spleen increased significantly ($P<0.05$); the level of Aβ and APP proteins decreased significantly ($P<0.05$); and the level of LRP1 protein increased significantly revealed by immunohistochemical detection ($P<0.05$). Electro-acupuncture can improve the structure of hippocampal neuron, increase the viscera index of the brain and spleen, decrease the expression levels of Aβ、APP proteins, and this processed were realized by up-regulating the expression level of LRP1 protein.

Key words: Morphology, Protein Expression, LRP1

INTRODUCTION

Alzheimer disease (AD) is a common disease of old age. The patients of AD showed impaired learning and memory ability. The hippocampal tissues of AD patients appear to have the senile plaques whose main component is amyloid beta protein (Aβ) [1]. There are many hypothesizes about the nosogenesis of AD. One of the hypothesizes is the neurovascular hypothesis proposed recent years. The hypothesis proposed that the basic structural unit of brain tissues is neurovascular unit including capillary endothelial cells, astrocytes, pericytes, neuron and vascular basement membrane; it was considered that AD was caused by the increase abundance of Aβ in the brain tissue; the ability of blood brain barrier eliminating Aβ was reduced which cause the Aβ accumulated significantly; and this further caused the vascular lesions worse, inflammatory reaction, and the blood brain barrier was further damaged;

meanwhile, the expression of related proteins on blood brain barrier were changed [2]. The production and clearance of Aβ is a kind of dynamic equilibrium, if the clearance of Aβ was reduced or the production of Aβ was increased, the Aβ protein will be greatly accumulated. Aβ was produced from the precursor protein APP which can be processed by the α、β、γ secretase to produce $Aβ_{1-40}$ and $Aβ_{1-42}$, $Aβ_{1-42}$ has stronger neurotoxicity than $Aβ_{1-40}$[3]. The clearance of Aβ is mainly depend on a pair of transporter existed on the blood brain barrier, which include advanced glycation end products receptor (RAGE) and low density lipoprotein related protein receptor 1 (LRP1) [4]. LRP1/RAGE receptor system plays important roles in the clearance of Aβ on the blood brain barrier [5]. During the process of Aβ clearance, Aβ firstly binds to the ligand of LRP1 such as apolipoprotein J (ApoJ), apolipoprotein E (ApoE) and $α_2$-macroglobulin complex formation which was recognized by the LRP1 of vascular endothelial cells to export the soluble Aβ protein from the brain [6,7]. The research indicated that electro-acupuncture have a therapeutic effect on AD, by bilat-eralm Shenshu, Neiguan and Dazhui can improve the learning, memory and activity of rats [8]. Based on these results, this study take advantage of electro-acupuncture to interment on mice with AD, to further study the relations of AD and LRP1 signal transduction pathway, and to explore the effect of electro-acupuncture on hippocampal neurons, by the objective evaluation of the curative effect of electro-acupuncture, this study aims to target of evaluation factors closely related to treatment with AD, and provide the basis for the study on therapeutic effect of electro-acupuncture.

EXPERIMENTAL SECTION

2.1 Materials

Anti-LRP1（Beijing Biosynthesis Biotechnol，bs-2677R; Aβ and APP ELISA Kit（Nanjing Jiancheng Bioengineering Institute 20140401）; $Aβ_{1-42}$（Sigma）; piracetam (Hunan Dinuo Pharmaceutical Co., Ltd 130325); C57 mice (Beijing Weitong Lihua Experimental Animal Technology Co., Ltd.), license number SCXK (Jing 2012-0001); other reagents are domestic pure analysis.

2.2 Equipment

Leica-2135 microtome (German Leica company)，TGL-16G desk centrifuge (Shanghai Anting Scientific Instrument Factory)，PLZOZ-S electronic balance (Mettler Toledo Instruments Co., Ltd.)，6100 type RT- ray enzyme mark instrument (American RT company)； electronic balance (Shenyang Longteng electronic weighing instrument Co., Ltd.).

3 Method

(1)Model preparation

After anesthetizing the mice by chloral hydrate, inject 5 μL condensation $Aβ_{1-42}$ into the ICV by the microsyringe ($Aβ_{1-42}$80pmol•μl), and leaving the needles for 3 mines; dressing the mice in sterile conditions. For the control group, inject equal saline.

(2)Grouping and Administration

After the model preparation, the SD mice were randomly divided into four groups including control group, model group, treatment group and electro-acupuncture group, 7 mice in each group. For the control group and model group, given normal saline to intragastric administration; for the treatment group, given 0.62 $g•kg^{-1}$ piracetam to intragastric administration; and for the electro-acupuncture group, using electro-acupuncture to bilat-eralm Shenshu, Neiguan and Dazhui for 30min•d^{-1}, the total will last 30 days. The animals were free intake of food, drinking water, raised at room temperature and humidity.

(3) Observation hippocampal by HE staining

Preparation of the paraffin sections of mice conventionally which contains the steps of dewaxing, ethanol dehydration, hematoxylin staining, hydrochloric acid ethanol differentiation, treatment of ammonia water，eosin staining, ethanol dehydration, transparent, fair, toast, and mounting; then observe the organization structure of CA1 and CA3 of hematoxylin by optical microscopy.

(4) Observation hippocampal by transmission electron microscope

Choose the mice brain hippocampus, fix it by 2.5% glutaraldehyde, after osmium tetroxide fixed, ethanol dehydration, embedding and sectioning, then observe the organization structure of hippocampal CA3 by electron microscope.

(5) Organ index determination

Kill the mice by decapitating and dissected it rapidly on ice; take the brain, spleen out, wash them clean by the physiological saline; and then use electronic balance to measure the weight of these organs, and calculate the organ

index.

Organ index of mice = Organ weight (mg)/ Body weight of mice (g)

(6)Detect APP and Aβ by ELISA

Rapidly take out hematoxylin tissue after killing the mice by decapitating, homogenate it, static it at 4℃ for 1 hour, centrifuge it at 3000 r•min-1 under 4℃, then split chargin and store it in -20℃. Then, take operation according to the instructions of the ELISA kit to detect the absorbance (A) at 450nm of each pole.

(7)Detect LRP1 by immunohistochemical

Preparation of the paraffin sections of mice conventionally, and then by the process of microwave dewaxing, repair, H_2O_2 treatment, serum blocking, add the anti-body, washing, add the anti anti-body, and then observe the LRP1 protein by DAB coloring and record the number of positive cells in the hippocampus of brown cells, select 6 sections of each group for statistical analysis.

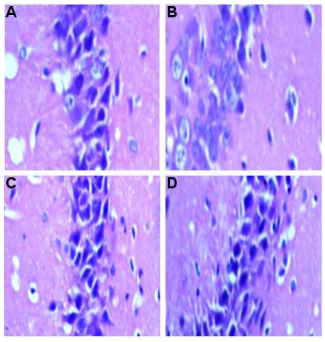
(8) Statistical analysis

Take advantage of SPSS19.0 software，the data was indicated by $\bar{x} \pm s$，data analysis among groups was conduct by single factor analysis of variance.

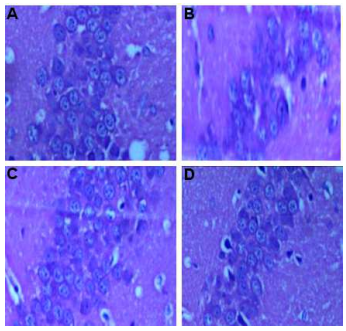
RESULTS

4.1 Observation CA_3 area of hippocampal by HE staining

It can be seen from figure 1, in the control group, the neuron in CA_3 area of hippocampal was well-distributed, three to four layers, closely packed and it has clear nucleus; in the model group, the neuron was only two to three layers with less cell numbers, the nucleus was pyknosised and disorder arranged; in the treatment group, there are little neuron shriveled, the cells was well-distributed with clear structure; and in the electro-acupuncture group, there are also little neuron shriveled, pyramidal cells were three to four layers with well-arranged.



(A. Control group；B. Model group；C. Treatment group；D. Electro-acupuncture group)
Figure 1 CA_3 area of mice hippocampal (HE staining，×400)



(A. Control group；B. Model group；C. Treatment group；D. Electro-acupuncture group)
Figure 2 CA_1 area of mice hippocampal (HE staining，×400)

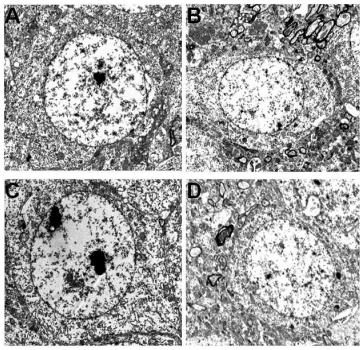
4.2 Observation CA_1 area of hippocampal by HE staining

It can be seen from figure 2, in the control group, the neuron in CA_1 area of hippocampal was well-distributed, three to four layers, and the nuclei, nucleoli and cytoplasm are clear; in the model group, the neuron was only two to three

layers with disorder arranged, and there are many cytoplasmic vacuoles; in the treatment group, the morphological changes were significantly improved, the neuron cells were three to four layers; and in the electro-acupuncture group, the neuron cells were also three to four layers with regular arranged.

4.3 Observation the neuron of CA_3 area of hippocampal by transmission electron microscope

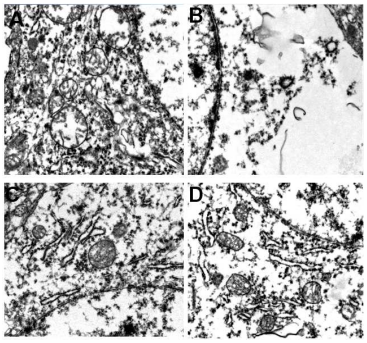
In the control group, the morphology of neuron was regular, the nuclei was clear, the cytoplasm was abundant; in the model group, he morphology of neuron was irregular and disorder arranged; in the treatment group, the morphology of neuron was regular, the structure was restored; in the electro-acupuncture group, the neuron of hippocampal was small and the morphology was regular. These results can be seen in figure 3.



(A. Control group；B. Model group；C. Treatment group；D. Electro-acupuncture group)
Figure 3 The neuron of CA_3 area of hippocampal (TEM，×4200)

4.4 Observation the neuron of CA_1 area of hippocampal by transmission electron microscope

The mitochondria in the control group was abundant and the golgi apparatus was clearly visible; the rough endoplasmic reticulum and golgi complexes less and the vacuoles was increased in the model group; the organelles were increased, and the structure was clear in the treatment group; and in the electro-acupuncture group, the organelles were increased and the rough endoplasmic reticulum was also increased despite some of them are swelling. These results can be seen in figure 3.



(A. Control group；B. Model group；C. Treatment group；D. Electro-acupuncture group)
Figure 4 The neuron of CA_1 area of hippocampal (TEM，×16500)

4.5 The influence of electro-acupuncture on the organ indexes of brain and spleen

The results indicated that compared with the control group, the organ indexes of the brain and spleen in the model group are significantly decreased ($P<0.05$); compared with model group, the organ indexes of the brain and spleen in the treatment group and electro-acupuncture group are significantly increased ($P<0.05$). These results can be seen in table 1.

Table 1. The influence of electro-acupuncture on the organ indexes of brain and spleen（$\bar{x}$ ±s，n=7）

Group	Dosage（g•kg^{-1}）	Spleen index（mg•g-1）	Brain indes（mg•g^{-1}）
Control group	-	2.99±0.46	8.02±0.34
Model group	-	2.40±0.45[1)]	7.47±0.26[1)]
Treatment group	0.62	2.73±0.26[2)]	7.88±0.28[2)]
Electro-acupuncture group	1.17	2.72±0.26[2)]	7.86±0.28[2)]

Notice：Compared with the control group [1)] $P<0.05$；compared with model group[2)] $P<0.05$

4.6 The influence of electro-acupuncture on the expression of hippocampal APP and Aβ proteins

The results indicated that compared with the control group, the expression levels of APP and Aβ were significantly increased ($P<0.05$); compared with model group, the expression levels of APP and Aβ were ignificantly decreased in the treatment group and electro-acupuncture group ($P<0.05$). These results can be seen in table 2.

Table 2. The influence of electro-acupuncture on the expression of hippocampal APP and Aβ proteins

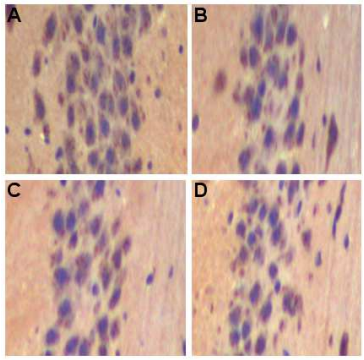
（$\bar{x}$ ±s，n=7）

Group	Dosage（g•kg^{-1}）	Hippocampal APP（ng•L^{-1}）	Hippocampal Aβ（ng•L^{-1}）
Control group	-	436.21±117.81	307.30±67.54
Model group	-	630.12±136.26$^{1)}$	385.75±55.26$^{1)}$
Treatment group	0.62	488.55±107.26$^{2)}$	306.62±51.71$^{2)}$
Electro-acupuncture group	1.17	482.79±83.51$^{2)}$	311.03±53.50$^{2)}$

Notice：Compared with the control group $^{1)}$ $P<0.05$；compared with model group$^{2)}$ $P<0.05$

4.7 The influence of electro-acupuncture on the expression of hippocampal LRP1 protein

The staining of hippocampal LRP1 protein in the control group was +～++, and there are many brown granules; the staining of hippocampal LRP1 protein in the model group was -～+, its expression level was obviously reduced, and the brown granules was very little; the staining of hippocampal LRP1 protein in the treatment group and electro-acupuncture group were +～++, and there are also many brown granules. These results can be seen in figure 5.



(A. Control group；B. Model group；C. Treatment group；D. Electro-acupuncture group)

Figure 5. The influence of electro-acupuncture on the expression of hippocampal LRP1 protein (immunohistochemical ×400)

The statistical results showed that compared with the control group, the positive neurons cell number in the hippocampal was obviously reduced in the model group; compared with model group, positive neurons cell number in the hippocampal of treatment group and electro-acupuncture group were significantly increased ($P<0.05$). These results can be seen in table 3.

Table 3. The influence of electro-acupuncture on the expression of hippocampal LRP1 protein（$\bar{x}$ ±s，n=7）

Group	Dosage（g•kg^{-1}）	No. of positive cells
Control group	-	15.71±2.56
Model group	-	10.43±2.64$^{1)}$
Treatment group	0.62	14.14±2.19$^{2)}$
Electro-acupuncture Group	1.17	14.57±2.23$^{2)}$

Notice：Compared with the control group $^{1)}$ $P<0.05$；compared with model group$^{2)}$ $P<0.05$

DISCUSSION

Electro-acupuncture has obvious effect on the improvement of AD animal model [9]. The research about electro-acupuncture with C57 background mice established experiment research of AD model which is still belongs to the blank so far. And at present, the research about the new AD theory- neurovascular hypothesis about the neurovascular unit closely related factor LRP1 is very little. Thus, this study developed the research about the effect of electro-acupuncture on the morphology and LRP1 protein expression in the model mice with AD.

This study was about the preventive and therapeutic effect of bilat-eralm Shenshu, Neiguan and Dazhui electro-acupuncture on the AD model mice. Observed by the optical microscopy, it has revealed that in the model

group, the neuron in CA_3 and CA_1 areas of hippocampal were two to three layers, the arrangement were disordered, and there are many cytoplasmic vacuoles which indicated that the model was successfully established and the model was stable. After electro-acupuncture treatment, the neurons of hippocampal were three to four layers and regular shaped; the nuclei, nucleoli and cytoplasm are clearly to be seen which indicated that there is obvious effect of electro-acupuncture treatment.

It has been reported that the CA_3 area of hippocampal is closely related to learning and memory [10], which remind us to detect the ultra-structure of hippocampal CA_3 area. The result showed that in the model group, the neurons were irregular shaped, disorder arranged; and the endoplasmic reticulum and golgi complexes were reduced which indicated that the hippocampal area of model group mice was severely damaged; after electro-acupuncture treatment, the shape of neurons became regular, the structure were also restored, the organelles were increased, and the endoplasmic reticulum was also increased which indicated that electro-acupuncture treatment recovered the structure of hippocampal CA3 area to different extents. Further morphological observation suggested that electro-acupuncture treatment have preventive and therapeutic effect on AD mouse and practical value which provide a theoretical basis for the clinical treatment.

Organ indexes were detected in all group mice, the results showed that compared with the control group, the organ indexes of the brain and spleen in the model group are significantly decreased ($P<0.05$) which indicated that the in the model group mice the of spleen and brain are quality declined and hypokinetic; compared with model group, the organ indexes of the brain and spleen in the treatment group and electro-acupuncture group are significantly increased ($P<0.05$) indicating that electro-acupuncture treatment can increase the organ indexes, partially restorethe function of the brain and spleen which also has a certain effect on the recovery of AD mice anti-inflammatory and brain function.

This study also measured the content of APP and its product Aβ by enzyme-linked immunosorbent assay which is that by the color reaction of the substrate to determine whether the corresponding immune reaction happened, and the depth of the color was proportional to the proportional. The results showed that compared with the control group, the expression levels of APP and Aβ were significantly increased ($P<0.05$) indicating that the key link of AD pathogenesis components -APP and A β are higher in the model mice brain tissue, the model is stable, and the subsequent experiments can be conducted; and compared with model group, the expression levels of APP and Aβ were ignificantly decreased in the treatment group and electro-acupuncture group ($P<0.05$), which also indicated that electro-acupuncture treatment has an improvement effect on AD.

According the neurovascular hypothesis, we detected the content of one of the Aβ transporters on the blood brain barrier-LRP1 protein. the results showed that hippocampal LRP1 protein in the model group mice was -～+, the positive neurons cell number in the hippocampal was obviously reduced ($P<0.05$), s expression level was obviously reduced, and the brown granules was very little; hippocampal LRP1 protein in the electro-acupuncture group were +～++, there are many brown granules, and positive neurons cell number in the hippocampal was obviously increased ($P<0.05$) which suggested that electro-acupuncture treatment has obvious therapeutic effect, meanwhile, it can also conclude that LRP1 is one of the important target of molecular mechanism in electro-acupuncture treatment of AD.

In summary, electro-acupuncture treatment of bilat-eralm Shenshu, Neiguan and Dazhui can obviously improve the brain function and significantly reduce the degree of aging mice brain. It also has a obvious improvement effects on brain morphology, meanwhile it can up-regulate the expression level of LRP1 protein obviously. These results suggested that the preventive and therapeutic effect of electro-acupuncture treatment of bilat-eralm Shenshu, Neiguan and Dazhui is realized by up-regulate the protein level of LRP1. However, the detailed mechanism needs further experimental study to uncover.

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