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Effect of Taurine in Combination with Electroacupuncture on Neuronal Damage Following Transient Focal Cerebral Ischemia in Rats

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ABSTRACT:

The present study was to investigate whether taurine is beneficial to the neuroprotective effects of electroacupuncture (EA) on rat cerebral ischemia induced by transient Middle Cerebral Artery Occlusion (MCAO). Histological change and DNA damage degree were detected by H&E staining and TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) method. Taurine and EA combination group could significantly decrease the percentages of infarct area and cell loss induced by ischemic injury in the striatum ($p<0.05$, vs singly treated with taurine or EA), and also the percentage of TUNEL-positive cells was smaller than both singly treated groups ($p<0.05$). Moreover, immunoreactivities detection of Bcl-2 (which promotes cell survival), Bax (which promotes cell death) and P53 (product of tumor suppressor gene p53) showed that the combination group apparently attenuated P53 immunoreactivities expression whereas augmented the immunoreactivities expression ratio of Bcl-2 to Bax in cortex. These results suggested that combining use of taurine and EA could exert better neuroprotective effects, which might be related to the regulation of apoptosis pathway.

KEY WORDS: Electroacupuncture (EA); Taurine; Cerebral ischemia; Neuroprotection.

INTRODUCTION

Taurine, one of the inhibitory amino acids (IAA) in brain, exerts extensive cell protection. It has been reported to protect ischemic heart cell from injury, due to its beneficial effects of augmenting cardiac muscle contractibility, attenuating membrane lipids peroxidation, inhibiting intracellular calcium overload and sodium boost etc [1]. Our previous studies observed enhanced release of taurine during cerebral ischemia / reperfusion and showed that electroacupuncture (EA) could further enhance the rise of extracellular taurine, which indicated the possible endogenous protective effect of taurine against excitatory toxicity [2]. Since EA is a good therapeutic means for cerebral ischemia, as is proved by considerable clinical cases and basic experiments as well [3,4]. Then a question rose: what's the role of taurine in EA neuroprotection? In this study, we tried to investigate whether taurine is beneficial to the neuroprotective effects of electroacupuncture (EA) on rat cerebral ischemia induced by transient Middle Cerebral Artery Occlusion (MCAO).

MATERIALS & METHODS

Animal Preparation

Adult male Sprague-Dawley rats from the Experimental Animal Center of Chinese Academy of Science were divided into 5 groups: ischemia (n=7); ischemia + saline (n=8); ischemia + taurine (n=8); ischemia + EA (n=8); ischemia + taurine +EA (n=7).

Focal cerebral ischemia was produced with a 4-0 nylon suture inserting to the origin of middle cerebral artery. After 2 hours occlusion, the suture was withdrawn to accomplish reperfusion.

EA was given to the points "Ren Zhong"(GV 26) and "Bai Hui" (GV 20) 15 minutes after MCAO by G-6805-2 EA Apparatus (Shanghai Medical Electronic Apparatus Company, China), lasting for 1 hour with a 10 minutes stop every 30 minutes. The waveforms of EA was dense-sparse, with the parameters of dense waves: Frequency 18 HZ, Intensity 1.8-2.5 mA, Duration 1.05 sec; sparse waves: Frequency 3.85 HZ, Intensity 1.4-2.5 mA, Duration 2.85 sec. (Fig 1).

Taurine (80 mg/Kg, iv, Sigma) was injected 30 minutes before ischemia. Saline was given as a control with the same way.

H&E staining and TUNEL method

After 24 hours reperfusion, rats were sacrificed. Each brain was removed and paraffin embedded. 6 μm coronal sections were cut and thaw-mounted on poly-L-lysine coated glass slides. The series of sections from bregma –0.26 mm were used for H&E staining to observe histologic changes. Another series of the adjacent sections were for TUNEL method to detect in situ DNA fragmentation. The procedure was carried out according to the "In Situ Cell Death Detection Kit,

AP" instruction mannual (Boehringer Mannheim). In brief, first dewax and rehydrate tissue sections according to standard protocols. Incubate with proteinase K (20 μg/ml in 10mM Tris/HCl, pH 7.4 for 15-30 min at 37°C). Rinse slides twice with PBS. After that, add 50 μl TUNEL reaction mixture on samples and incubate in a humidified chamber for 60 min at 37°C. Rinse slides twice with PBS. Add 50 μl Converter-AP(alkaline phosphatase). Incubate in a humidified chamber for 30 min at 37°C. Rinse slides three times with PBS. Add 50 μl substrate solution (NBT/BCIP). Incubate slides for 20 min at room temperature. Rinse slides with PBS. Analysis by light microscope.

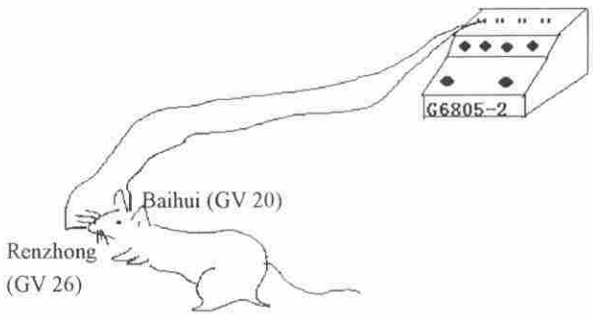

Fig.1 Schematic diagram showing the electroacupuncture procedures in rat with EA apparatus (model G6805-2).

Immunohistochemistry for Bcl-2, Bax and P53 proteins

Other three series of sections adjacent to bregma –0.26 mm were treated to study the expression of Bcl-2, Bax and P53 proteins, with antibodies from Santa Cruz Company(1:100 polyclonal IgG for Bcl-2 and P53, 1:100 monoclonal IgG for Bax). The immunoreactivity was detected by DAB.

Infarct area measurement and cell number counting

Infarct and ipsilateral hemisphere area of each H&E stained section were calculated using Leica Q500 IW Image Processing System. Lesion extend was presented by the percentages of infarct area versus ipsilateral hemisphere area.

Number of residual cells, TUNEL-positive cells and immunoreactive-positive cells were counted under a high power microscope (10x20) and recorded as the number of cells per high power field (HPF). Cell loss degree was expressed by the percentages of the number of ipsilateral residual cells versus that of contralateral cells. DNA fagmentation extend was diaplayed as the ratios of TUNEL-positive cell

number versus ipsilateral residual cell number.

Data analysis and statistics

All data were presented as mean ± SD. Data comparison was assessed by student's t-test. Differences of $p<0.05$ was considered as significant.

RESULTS

Lesion extend

As seen in Tab.1, lesion extend in "EA + tau + isc" group was smaller than that both in "EA + isc" group and "tau + isc" group ($p<0.05$) though the later two groups showed significant differences ($p<0.05$) from ischemia group.

Cell loss degree

In striatum, cell loss degrees were more severe than that in cortex in all the five groups (Tab.1). Residual cell number in "EA + tau + isc" group was the largest. Differences between "EA + tau +isc" group and "EA + isc"group or "EA + tau +isc" group and "tau + isc" group were both significant ($p<0.05$).

DNA fragmentation degree

TUNEL-positive cells could be clearly seen in ipsilateral cortex and striatum, whereas in contralateral side, only few could be found. In cortex, no differences were calculated among "EA + isc", "tau + isc" and "EA + tau + isc" group, though DNA damage degree in the later group seemed smaller than that in the former two groups. There were significant differences ($p<0.05$) in striatum of "EA + tau + isc" group from both "EA + isc" and "tau + isc" group (Tab.1).

Tab.1 Effect of single or combining use of EA and taurine on lesion extend, cell loss degree and TUNEL (+) degree

	Infarct area (%)	residual cell (%)		TUNEL (+) (%)	
		cortex	striatum	cortex	striatum
ischemia	61.35±4.26	87.57±5.34	57.96±9.8	19.72±1.72	87.05±11.79
EA+isc	41.28±3.32 *	96.89±4.68	72.8±8.1 *	4.23±1.62 **	64.52±12.34 *
saline+isc	62.12±13.11	91.42±4.23	55.76±10.31	14.56 ± 4.57	77.12 ± 8.14
tau+isc	34.56±12.28#	97.35±5.21	73.84±9.24#	3.09 ± 1.20##	59.74 ± 6.44#
EA+tau+isc	20.23±4.33 •§	97.43±4.54	83.3±8.4 •§	2.87±1.12	48.88±5.73 •§

* $p<0.05$, ** $p<0.01$ *vs* ischemia group

# $p<0.05$, ## $p<0.01$ *vs* saline control group

• $p<0.05$ *vs* EA+isc group

§ $p<0.05$ *vs* tau+isc group

Bcl-2, Bax and P53 like immunoreactivities expression

In striatum of all the five groups, few Bcl-2-like immunoreactive cell was seen. Though P53-like and Bax-like immunoreactive cells could be found in ipsilateral striatum, differences were not significant among the five groups (data were not shown). In penumbra cortex, the ratio of Bcl-2/Bax rose from 1.41 ± 0.03 in "EA + isc"group and 1.68 ± 0.18 in "tau + isc" group to 2.12 ± 0.23 in "EA + tau + isc"group (P<0.05), (Fig. 2). As for the expression ratio of P53 in penumbric cortex, it declined the most in "EA + tau + isc" group (P<0.05), (Fig. 2).

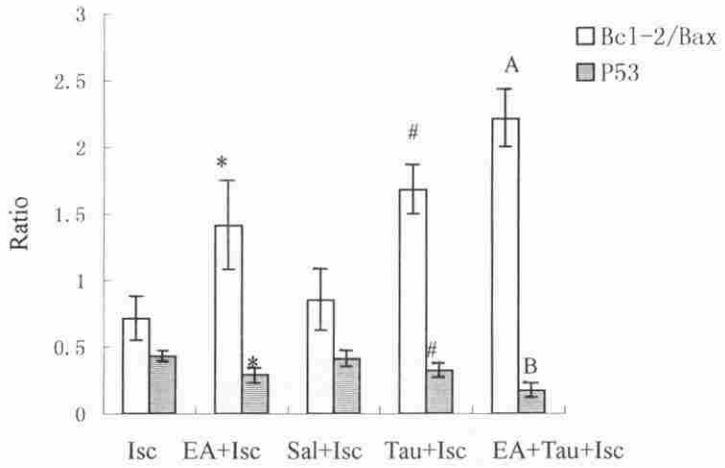

Fig.2 Expression ratio of Bcl-2/Bax and P53 like immunoreacitvities in the ipsilateral cortex.
Isc: ischemia; EA: electroacupuncture; Tau: taurine; Sal: saline.
*: P<0.05 compared with ischemic group;
#: P<0.05 compared with saline group;
A: P<0.05 compared with EA group;
B: P<0.05 compared with both EA group and Tau group.

DISCUSSION

TUNEL (TdT-mediated dUTP nick end labeling) method was also described as ISEL (in situ end labeling). The procedure is based on the in situ-labeling of DNA fragmentation. Cleavage of genomic DNA during apoptosis and necrosis may yield double-stranded as well as single stranded breaks. The free 3'-OH termini of those DNA strand breaks can be identified by

TdT (terminal deoxynucleotidyl transferase), which catalyzes polymerization of nucleotides to free 3'-OH DNA ends in a template-independent manner. Since TUNEL method could label both double and single strand fragmentation of nucleosomal DNA and non-nucleosomal DNA, so it was used as a marker of DNA damage degree in this study [5]. The present work confirmed that taurine supplement could exert a better neuroprotection of EA on cerebral ischemia since the combination group could further lessen the lesion extend, reduce cell loss number and alleviate DNA damage degree. It is reported that taurine was applied in the treatment of cardiovascular disease and had little side effects [6] Our result also indicated a potential clinical application of taurine combining with EA in stroke treatment.

As an inhibitory amino acid extensively located in brain, taurine is known to be involved in the processes including modulation of cellular calcium flux and neuronal excitability. Our study indicated that treated with taurine alone also protect brain against neural injury. These results were consistent with other reports, which suggested its possible neuroprotection in neural damage conditions [7-9]. To our interesting, the neuroprotection degree of taurine is very similar with that of EA. Considering the fact that EA could further enhance taurine release during ischemia / reperfusion [2], we tried to elucidate the possible mechanisms. In our experiment, we observed that both taurine and EA could modulate the expression of apoptosis-related proteins, which might inhibit apoptosis pathway. Moreover, our previous work reported EA could attenuate the extracellular level of excitatory amino acids [10] and nitric oxide [11] detected by microdialysis-HPLC and by in vivo NO-sensitive electrode respectively. On the other hand, some researchers observed that taurine also could alleviate the presynaptic release of EAA [12]; and in our recent work we observed that taurine had the same effect on extracellular NO level as EA. Taken together, we noticed there exists some common pathways in EA and taurine neuroprotection effect. Thus we may suppose that the enhanced release of taurine might play an important role in the mechanisms of EA neuroprotection, that is, taurine might partly mediate the neuroprotective effect of EA. To confirm this suppose, some further researches are needed.

ACKNOWLEDGEMENT

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We have made the following revisions:

1. Peng Zhao is an assistant professor.
2. Professor Jieshi Cheng is M.D..
3. After the institutional affiliation, we added:" Fax:86-21-64174579, email: jscheng@shmu.edu.cn."
4. "Key Words" was revised to "KEY WORDS".
5. In abstract text, we added "(terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling)" after the word "TUNEL".
6. In "MATERIALS & METHODS", we added a paragraph "In brief,..." after the "H&E staining and TUNEL method" section. This paragraph mainly described the procedure of TUNEL.
7. In "DISCUSSION", we added some information of TUNEL in the first paragraph.
8. We added one reference to further describe TUNEL method. The reference number is FIVE. So, the No. 5 reference in the previous manuscript was changed to No.6. The previous No. 6 was changed to No. 7....