

Effects of estrogen on learning-memory and expression of calbindin-D28K in hippocampus in vascular dementia rats

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Abstract: Vascular dementia (VD) models were made first by repeating cerebral ischemia-reperfusion and followed by treating with estrogen. Learning-memory ability was measured by Morris water maze. Concentration of Ca²⁺ in hippocampus was determined by Fura-2/AM fluorescence probe and the expression of Calbindin-D28K (CB) in hippocampal CA1 was tested by immunohistochemistry. Learning-memory ability was improved in E group rats; Concentration of Ca²⁺ in hippocampus was decreased in E group rats. The expression of CB was less in E group rats. It implies that estrogen could improve learning-memory ability in VD rats, which may be associated with suppressing intracellular Ca²⁺ overload and increasing the expression of CB in hippocampus.

Keywords: Vascular dementia, Learning- memory, Hippocampus, Calbindin-D28K

INTRODUCTION

VD is one of the main diseases that has threaten the elderly health and quality of their life, but its pathogenesis is not fully clear, and its clinical interventions is lack of specificity (Fernandez Martinez *et al.*, 2008). Estrogen has a strong hydrophilic fatty, it can easily penetrate the blood brain barrier into the brain and act on its receptors. The main functions of hippocampus are information storage, memory and spatial orientation, which is the most sensitive to ischemia and hypoxia (Zhang *et al.*, 2012). The core of the pathogenesis of VD is that cerebral ischemia destroy the loop structure of learning and memory (such as the hippocampus). It is shown that there are abundant estrogen receptors in hippocampus (Mehra *et al.*, 2005, Allahtavakoli *et al.*, 2015, Shiga *et al.*, 2016). Endogenous estrogen could be synthesized in the brain, which can adjust synaptic plasticity related to memory. Researches show that the physiological doses of estrogen is related to learning and memory (Li and Shen, 2005). However, whether estrogen could improve learning and memory dysfunction and how to improve it in VD rats is not clear. Therefore this thesis will discuss the possible mechanisms of estrogen on VD rats.

MATERIALS AND METHODS

The experiment was conducted at the Medical College of Beihua University from March 2014 to June 2016.

Animals

Wistar male rats aged 10-12w, were purchased from Laboratory Animal Center of Jilin University (Certification No. SCXK (Ji)2007-0003). Animals were

housed in standard temperature (22±1°C), humidity (40-50%) and 12h light/dark cycle. All animals were housed in the facility under these conditions for 1 week prior to experiments. Rats were randomly divided into normal (N), sham-operation (S), model group (M) and E group. 10 in each group.

N group: There was no any processing.

S group: Rats were only separate common carotid artery, set of silk buckle, but not block blood flow.

M group: Rats were make VD model, place sesame oil on subcutaneous back (inside/outside diameter is 1.575/3.175 mm, length is 20mm).

E group: Rats were make VD model, place estrogen capsule on subcutaneous back (inside/outside diameter is 1.575/3.175 mm, length is 20 mm, 180µg/ml).

VD Model

Rats were intraperitoneally injected 10% chloral hydrate (3.5 ml/kg) for anesthetization, lied on the operating table, disinfected, then cut in the neck. Bilateral common carotid arteries were Bilateral isolated; sodium nitrate is then injected to peritoneal cavity (2.5mg/kg). Bilateral common carotid arteries were ligated by thread for 10 min to block blood flow. The thread was loosened to allow blood reperfusion for 10 min. The above procedures were repeated in triplicate to establish models of VD.

Apparatus and reagents

Morris water maze was purchased Taimeng; Fluorescence spectrophotometer was purchased from Hitachi; Estrogen was obtained from Sigma Aldrich; Calbindin D28K-kit was purchased from boster biological engineering co., LTD; Fura-2/AM was purchased from Beijing Bo Leide Biotechnology.

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Morris water maze

Morris water maze was a diameter of 120cm and height of 50cm circular water tank. It was equally divided into 4 quadrants (A, B, C, D). Diameter of 10cm platform was fixed in one of quadrant. The water surface was higher than the platform 1.5cm. Rats were trained to find platform for 6 days. Latencies were recorded in each trial. The animals needed to find the platform within 120s. Time and number of crossing platform were record.

Concentration of Ca^{2+} test by Fura-2/AM

Samples were loaded by Fura-2/AM at 37°C 30min, centrifuged 11000×g for 10min, determined by dual wavelength. The emission wavelength was 509nm and excitation wavelength was 340nm and 380nm. Fluorescence spectrophotometer was applied to measure resting fluorescence.

CB-positive neurons analysis

After the water maze test, the rats' hearts were perfused, and the rats were quickly sacrificed by decapitation to take out the brains and soaked them in 10% neutral formalin, dehydration, paraffin embedding, coronary section (8µm). CB staining procedures carried out in

accordance with the kit instructions. The mean gray value of CB staining in sections was determined by analysis of randomly selected fields and quantification with IPP6.0 software.

STATISTICAL ANALYSIS

The data was analyzed by SPSS16.0. and presented with mean ± standard deviation (SD). Analysis by one-way analysis of variance. *Post Hoc Tests* was applied to sort statistically significant differences within groups.

RESULTS

Results of Morris water maze

Latency was longer in M than N group rats ($P < 0.05$, $P < 0.01$). After estrogen treatment, latency was shorter than M group rats ($P < 0.01$) (fig. 1-A). Time and number of crossing platform was no difference between S and N group rats ($P > 0.05$), but significantly decreased in M than N group rats ($P < 0.01$), significantly increased in E than M group rats ($P < 0.01$) (fig. 1-B, C).

Concentration of Ca^{2+} in hippocampal neurons

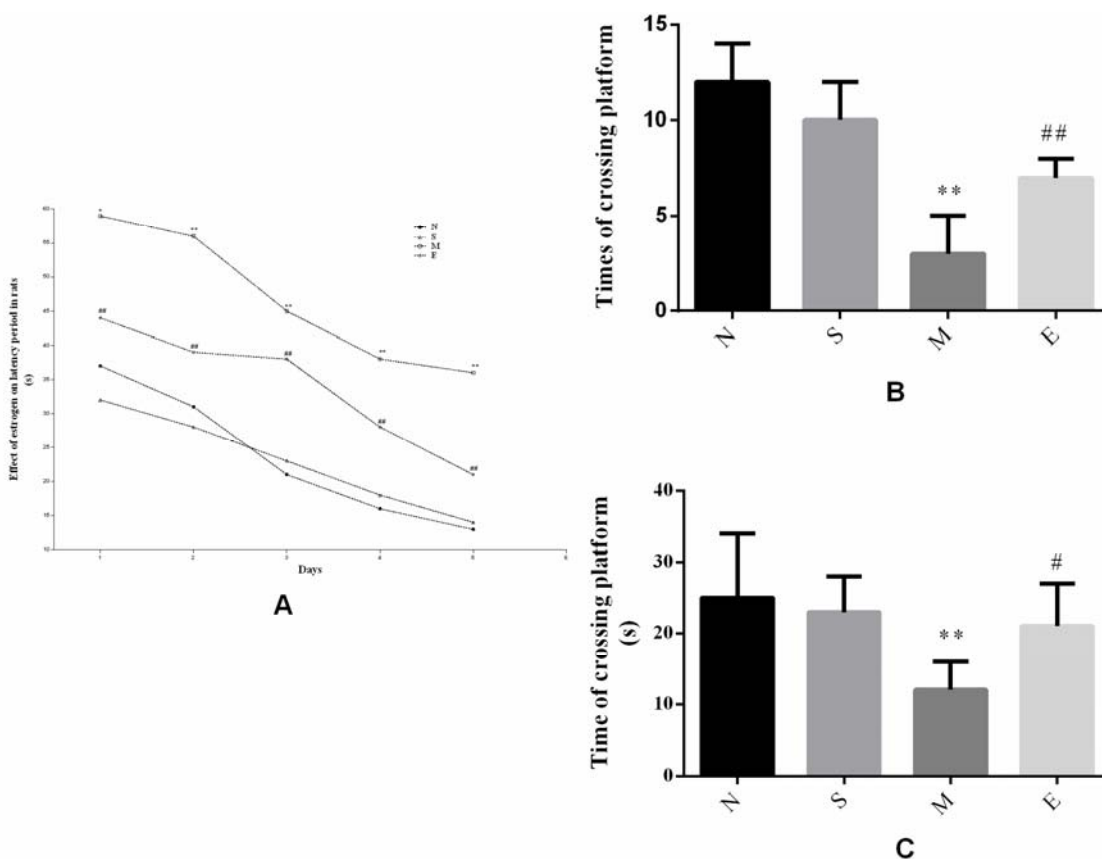


Fig. 1: Results of Morris water maze. A: n=10 animals in each group. Compared with N group, ** $P < 0.01$, * $P < 0.05$; Compared with M group, ## $P < 0.01$. B: n=10 animals in each group. Compared with N group, ** $P < 0.01$; Compared with M group, ## $P < 0.01$, # $P < 0.05$. C: n=10 animals in each group. Compared with N group, ** $P < 0.01$; Compared with M group, ## $P < 0.01$, # $P < 0.05$.

There was no difference between N and S group rats ($P>0.05$). $[Ca^{2+}]$ in hippocampus was higher in M than N group rats ($P<0.01$), was significantly reduced in E than M group rats ($P<0.01$) (fig. 2).

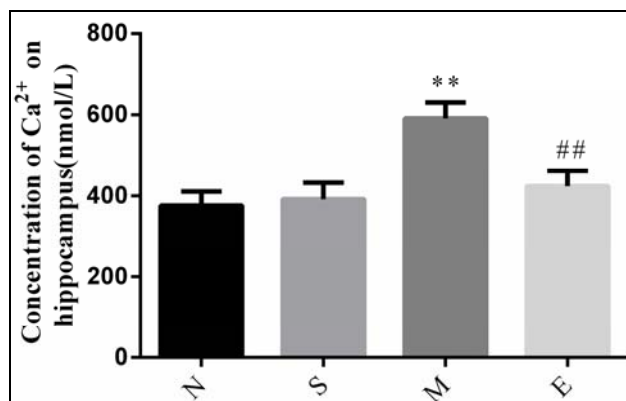


Fig. 2: Concentration of Ca^{2+} in hippocampal neurons. $n=10$ animals in each group. Compared with N group, ** $P<0.01$; Compared with M group, ## $P<0.01$.

CB-positive neurons

The number and average gray value of positive CB neurons in hippocampus were no significant difference in S and N group rats ($P>0.05$), was obviously decreased in M than N group rats ($P<0.01$), was obviously increased in E than M group rats, ($P<0.01$) (fig. 3-A, B)

DISCUSSION

Morris water maze is an important research method for spatial learning and memory, which can accurately reflect the ability of spatial learning and memory (Barnhart *et al.*, 2015). This study found that VD rats had a spatial cognitive impairment by Morris water maze test, which was consistent with other studies (Li *et al.*, 2013, Ge *et al.*, 2015, Li *et al.*, 2016), while estrogen significantly relieved cognitive impairment.

Ca^{2+} overload is considered to be the final route of neuronal cell death (Choi, 1994, Ghosh and Greenberg, 1995, Hyrc *et al.*, 1997). Ca^{2+} overload is closely related with cognitive dysfunction. The experiment results show that $[Ca^{2+}]$ significantly rise in VD rats, which shows that ischemia reperfusion causes Ca^{2+} overload, and leads to cognitive impairment. $[Ca^{2+}]$ is significantly lower after treatment with estrogen. It shows that the reason for estrogen inhibits ischemia-reperfusion brain damage is closely to alleviate Ca^{2+} overload.

Calcium binding protein is a part of the intracellular calcium buffer (Miller, 1991). The reduced number of calcium binding protein is negative effects on the cell's survival. Calcium binding protein in neurons can be combined with free Ca^{2+} , prevent Ca^{2+} overload, prevent the excitatory toxic effects, protect neural effect. Intracellular calcium binding protein such as CB was

considered as cytoplasmic Ca^{2+} buffers (Miller, 1991, Baimbridge *et al.*, 1992, Chard *et al.*, 1993). This research showed that the number and average gray of CB was decreased. It showed that CB positive neurons was significantly reduced in VD rats. The above two indicators were significantly increased in group E, which showed that estrogen could promote CB expression in VD rats. Therefore estrogen can improve learning and memory ability in VD rats, inhibit Ca^{2+} overload, improve CB expression in hippocampus. Whether there are other mechanisms or not remains to further research.

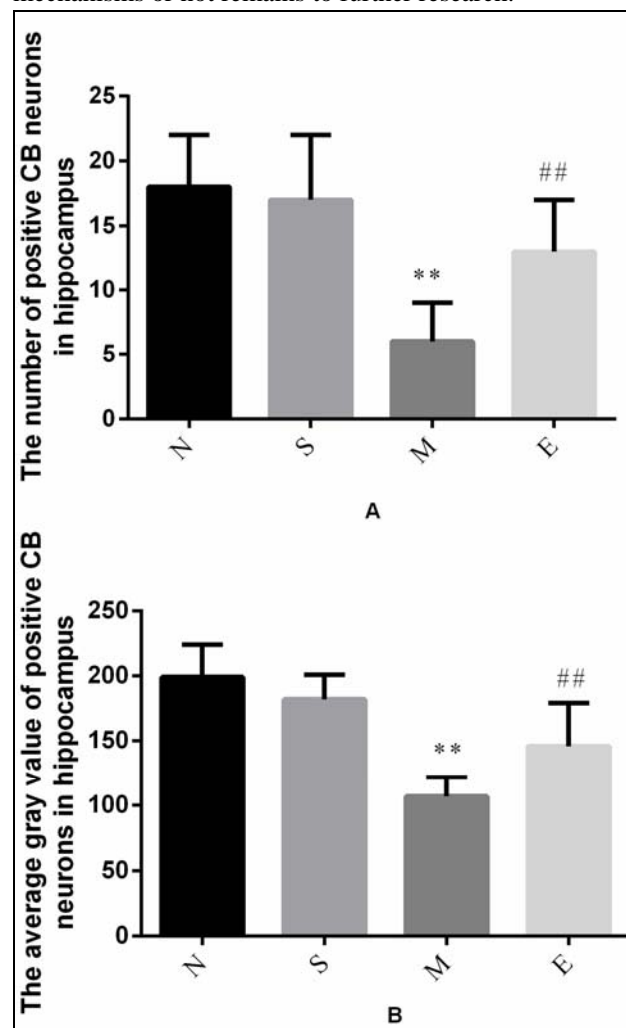


Fig. 3: The number and average gray value of positive CB neurons in hippocampus. A: $n=10$ animals in each group. Compared with N group, ** $P<0.01$; Compared with M group, ## $P<0.01$. B: $n=10$ animals in each group. Compared with N group, ** $P<0.01$; Compared with M group, ## $P<0.01$.

CONCLUSION

Estrogen could improve learning-memory ability in VD rats, which may be associated with suppressing intracellular Ca^{2+} overload and increasing the expression of CB in hippocampus.

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