Effect of schizandra B on reorganization of primary hippocampus neurons dendritic spine induced by epilepsy

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Abstract: To investigate the effect of schizandra B(Sch B) on reorganization of primary hippocampus cells dendritic spines induced by Glu. Methods: The primary hippocampus cells were divided into control group, model group and Sch B group. The primary cells in control group were untreated; the primary cells in model group were treated with Glu(15mmol.L-1)for 24h. The primary cells in Sch B group were firstly pretreated with Sch B（0.5μmmol.L-1）for 2h, and exposed to Glu(15mmol.L-1)for 24h. Results: A significant decrease of spines in number was found in the model group cells（3± 2.0/ 10μm n=12） when compared whose those in the control（12± 3.0/μm n=10，p<0.01,t-test）. A slightly decrease of spines in number were found in cells of Sch B group (8± 2.0/ 10μm n=12). Western blot analysis revealed that there is no effect on the total levels of actin and cofilin; However, it can decrease the levels of p-cofilin in the model group. The levels of p-cofilin in Sch B Group is higher than those in model group. Conclusion: Sch B can protect the damage of dendritic spines caused by Glu that is related to inhibit the activation of p-cofilin.

Patients with epilepsy often have cognitive impairment due to epilepsy itself [1-4]. Seizures can cause mild changes in dendritic morphology and dendritic spine of neurons. Dendritic spines is neuronal excitatory postsynaptic component. The number of dendritic spines of hippocampus and neocortex that is related to memory and cognition were decreased in the patient of epilepsy. We hypothesis abnormal dendritic spines maybe causes cognitive damage.[5-6].

The changes of the cytokines skeleton changes in glutamate activation are related to the seizure. Glutamate antagonist can prevent the decrease number of dendritic spines caused by seizures. Glutamate agonists kainic acid induced seizures cause the decrease in the number of dendritic spines in hippocampal CA1 area and the neocortex, which is caused by depolymerization of actin cytoskeleton cofilin [14]. At present, epilepsy is mainly controlled by antagonism of neurotransmitters or by blocking ion channels. This study attempts to treat epilepsy by stabilizing the intra-synaptic actin skeleton

Schisandra is a traditional Chinese medicine, which play roles in central inhibition and the role of tranquilization [15]. Studies have shown that schizandrine can prevent glutamate induced neuronal damage and antioxidation [10-11]. It not only effectively prevents the damage of the oxidative stress induced by glutamate in the rat brain, but also improves the memory function of mice by regulating the level of acetylcholine. It also promoted dendritic growth and synaptic formation in

cultured mouse cortical neurons. In vitro cultured hippocampal neurons was to explore the mechanism on the stability of cytoskeleton within dendritic spines induced by glutamate and provide the basis for the treatment of epilepsy.

Materials and Methods

Experimental animals, main reagents and instruments.

Sch B is purchased from Shanghai yuanye biotechnology company. NEUROBASAL MEDIUM, B-27 Supplement purchased from American GIBCO; Glutamate, polylysine and fetal bovine serum were purchased from Sigma company in the United States; Alexa-488 phalloidin antibody purchased from Invitrogen company; HRP labeled sheep anti-rabbit IgG, p-cofilin, cofilin, actin and Hoechst were all purchased from the American Sigma company; Western blot test was purchased from Thermo company with ECL kit (Enhanced Chemiluminescence Assay kit). Confocal microscope (FV1000, Olympus, Japan). The pregnant mouse (e16-18) is provided by the animal center of jilin university, and the animal certificate no. : SCXK-Ji (2006-0001).

Primary hippocampal neurons and cortical neurons were cultured .

The detailed culture method show in the paper [16]

Immunofluorescence assay for cultured hippocampal neurons in vitro.

abandon **cell media and** wash twice with 37 ˚C preheat PBS, the new **prepared** 4% pa**raformaldehyde fixe cells for** 20 min**utes**; Wash three times **with PBS**. 0.1**%** Triton X-100 **were added to cells** for 10 min**utes**; **cells were washed 3 times** and was **incubated for 30minutes with** 3% sheep serum at room temperature. **And then incubated** for the night **with Alexa Fluor 488 phalloidin conjugate (2 mg ml^{-1}) at** 4˚C. Cells were stained nucleus with Hoechst . Cells were examined with a laser scanning confocal microscope.

Western blot detection of cofilin, p-cofilin protein expression.

The cells were washed twice with Cold PBS, The homogenized samples were boiled for 5 min. The protein concentration was determined by the Lowry method. Thirty micrograms of protein were separated by 15% SDS-PAGE and transferred to polyvinylidene difluoride membranes. After incubation with a primary antibody (1:1000; Cell Signalling) that recognized phosphorylated cofilin (p-cofilin) at Ser3, the membranes were incubated with a peroxidase-conjugated secondary antibody and visualized with an ECL detection kit (Pierce). The blots were reprobedfor total cofilin (1:1000; Cytoskeleton) and actin (1:500; Sigma). The signals were scanned for quantitative analysis with ImageJ.

Statistical analysis was performed using SPSS17.0 statistical software. Randomly selected 10-15 neurons. The average number of dendritic spines and quantitative protein expression levels between the experimental group was used by t test, $p < 0.05$, with statistical significance.

Results

Morphological change of cell neuclei and dendritic spines.

The results showed that the nuclei of each group were normal and had no significant difference (the data was not shown). hippocampal neurons for 3 weeks were stained with Alexa Fluro 488 Phalloidin antibody (figure 2). Quantitative analysis of dendritic spine changes in control group, model group and schizandraB group. The number of dendritic spines in the model group was significantly reduced (**12± 3.0/ 10um** n=12) compared with the control group of dendritic spines (**3± 2.0/ 10um n=12**, n=10, p<0.01,t-test).The number of dendritic spines in schizandrine group was slightly decreased (**8± 2.0/ 10um n=12** n=12).

antagonist glutamine-induced cofilin activation by schizandra.

We used western blot to detect cofilin and p-cofilin at the protein level, and the results showed that the total actin and cofilin levels in each group were consistent, and the level of p-cofilin in the model group was decreased, while the level of the p-cofilin in the five MSG was higher than that in the model group.(see figure 1).

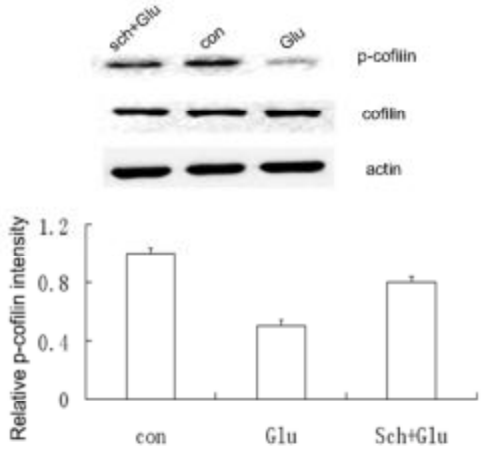

Figure 1 Ralative p-cofilin intensity was analysized by western blot between control model group and Schizandra. The ratio of p-cofilin to total cofilin is shown in histogram.(P<0.01, n=10, t-test).

Discussion

Changes in the number and morphology of dendritic spines are related to learning and memory[19-20]. Many scholars chose dendritic spine as the target to study the cognitive impairment caused by epileptic seizures. The density of the hippocampal dentate gyrus was reduced [13]. The number of dendritic spines in the CA1 region of the hippocampus was reduced [14]. Because of the changes of dendritic spine in specific parts of these brain regions, we chose to cultivate hippocampal neurons in low density in vitro. We use cortical neurons and hippocampal neurons to develop and solve the short survival time of individual neurons. The results showed that

cultured hippocampal neurons were cultured for 3 weeks and the neurons were in good condition, and the typical dendritic spine was seen in Phalloidin staining. Phalloidin is extracted from a only seven small dicyclic peptide, which can be inserted between the actin microfilament monomer, thus inhibiting the depolymerization of F-actin to keep skeleton composition stable in the tissues and cells[7]. Phalloidin tag is currently recognized as high specific F - actin detection method.

Glutamic acid is a kind of excited amino acid, can cause nerve excitability toxicity. Hubert et al found that too much glutamate could induce apoptosis in cultured cortical nerve cells in vitro and inhibit the growth of immature cortical neurons. In this study,

The current clinical treatment of epilepsy is mainly through the antagonism of neurotransmitter or blocking ion channels to suppress seizures of neural activity, but a third of the patients using existing treatments work hard. Schizandria can be treat epilepsy by stable synapse F-actin to suppress seizures and prevent cognitive impairment.

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