Repair Effects of NGF and FPS-ZM1 on brains of epileptic rats

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Research

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Abstract

Background Epilepsy in children seriously affects the quality of life and learning ability of children.And it is very important for repairing the brain function of children after seizure.In this study ,we will investigates the neuroprotective effects of NGF and FPS-ZM1 by measuring HMGB1of hippocampus and serum after intervening the rats with NGF and FPS-ZM1. It would provide theoretical basis for the treatment of brain injury caused by epilepsy.

Methods 130 Wistar male young rats were randomly divided into three groups: Group A (normal control group including 31 rats), group B (epilepsy group including 33 rats), group C (NGF group including 33 rats), group D (FPS-ZM1 group including 33 rats).9 living rats were randomly selected at 3hours,24hours and 72hours after the intervention from each group, blood samples and the hippocampus were taken .The expression of HMGB1 in hippocampus was measured by Western-blot, and the content of HMGB1 in serum was measured by enzyme-linked immunosorbent assay (ELISA).

Results The expression of HMGB1 in hippocampus and serum at each time point of group B was significantly higher than that of group A ($P < 0.01$). And there was no significant difference among of 3h, 24h and 72h. The expression of HMGB1 in hippocampus and serum of group C and D was lower than that of group B at each time point, the difference was statistically significant ($P < 0.05$). In group D, the expressions of HMGB1 in hippocampus and serum of group D were lower than that of group C at 3hours and 24 hours, the difference was statistically significant ($P < 0.05$), But there was no significant difference between group C and group D at 72 hours ($P > 0.05$).

Conclusion HMGB1 was significantly increased in hippocampus and serum of epileptic rats, it was suggested that HMGB1 was involved in the inflammatory process of epilepsy and HMGB1 could be used as a marker of brain injury degree after seizure. NGF and FPS-ZM1 had the effect of repairing the brain after seizure. FPS-ZM1 is superior to NGF in the protection of nervous system.

Introduction

Epilepsy is a common disease of the nervous system in children. Epilepsy in children seriously affects the quality of life and learning ability of children, and brings a heavy burden to their families and society. The mechanism of epilepsy is not fully understood. Repeated seizures cause great damage to the brain function of the children. So far, no marker has been identified or used to assess the degree of brain injury of epilepsy children. The ability to evaluate brain injury has become the focus of research. And it is very important for repairing the brain function of children after seizure. Therefore, in my study, the degree of brain injury was evaluated by observing the dynamic changes of high mobility group Box1(HMGB1) in hippocampus and serum of epileptic young rats. Subsequently, the study investigates the neuroprotective effects of NGF(Nerve growth factor) and FPS-ZM1 by measuring HMGB1of hippocampus and serum after intervening the rats with NGF and FPS-ZM1. It would provide theoretical basis for the treatment of brain injury caused by epilepsy.

Materials And Methods

Materials

Drug: FPS-ZM1 (USA, Meck Millipore, Lot: 2908726), dissolved in a small amount of DMSO (Beijing Ding Guo chang Sheng Biotechnology Co., Ltd.) and then diluted with saline, In the end,the concentration of DMSO was 1%.

Animals: 130 clean grade Wistar male rats, 3–4 weeks age, weighing 50–60 g, were purchased from Shanghai Sleek Laboratory Animal Co., Ltd., License No.: SCXK (Shanghai) 2017-0005.

Reagents: Pentylenetetrazol (USA, Sigma, lot: MKCC1690), was configured with saline the concentration of Pentylenetetrazol is 10 mg. ml-1. Western Blotting Assay Kit (KGP1201, Jiangsu Kaiji Biotechnology Co., Ltd.); photographic developer and fixative (KGP116, Jiangsu Kaiji Biotech Development Co., Ltd.); HMGB1 ELISA Assay Kit (Shanghai Langton Biotechnology Co., Ltd., Lot:S20180318M)

Method

Experimental group and intervention: 130 clean-grade Wistar male young rats were randomly divided into four groups: Group A including 31 rats (control group), group B including 33 rats (epilepsy group), group C including 33 rats (NGF group) and group D including 33 rats (FPS-ZM1 group).

The rats of groups B, C and D were injected with pentylenetetrazol(PTZ) to establish a model of chronic children epilepsy. After establishment of the model, the rats in groups A, B were injected with saline, and group C and group D were injected with NGF and FPS-ZM1 respectively every day for one week. 9 rats alive were randomly selected at 3hours,24hours and 72hours after the intervention from each group .All of rats were anesthetized by intraperitoneal injection of 2% pentobarbital at a dose of 40 mg/kg. Blood samples and the hippocampus of rats were taken.

The expression level of HMGB1 in hippocampus was measured by Western-blot (WB): The hippocampus tissues of rats were taken and the protein lysate was added, and homogenate was centrifuged at 10 000 r/min (4 ℃) for 5 min. Took the supernatant and selected 80V voltage for the electrophoresis. When the protein sample enters the separation gel (at this time the indicator is compressed into a line), increased the voltage to 120 V. And then transfered the membrane for 120 minutes with 200 Am constant electric current. Washed the membrane by Tris Buffered Saline Tween (TBST). Blocked them with 5% skimmed milk powder ,incubated them for 1.5-2 hours.

After membrane washing, added the primary HMGB1 antibody (1: 500), and GAPDH antibody (1: 10000) incubator, incubated at 4 ℃ overnight. washed membrane again. Added secondary antibody (Sheep anti-rabbit IgG (1: 5000)) and incubated them for 2 hours at room temperature. Washed membrane for the third time,developed, fixed, and analyzed the results with image processing system.

Determination of HMGB1 in serum by enzyme-linked immunosorbent assay (ELISA): Took serum samples of each group and measured them with ELISA kit. After adding sample, liquid preparation, washing and termination, measured the absorbance (OD) of each hole in sequence with the microplate reader at the wavelength of 450 nm.

Statistical Analysis

Statistical processing was performed using SPSS 24.0 software, and data were presented as mean ± standard deviation (). When the variance was homogeneous,One way ANOVA was used in the multiple means comparison .When the variances was uneven,LSD-t test was used to the pairwise comparison, Dunnett T3 test was used to multiple means comparison. $P < 0.05$ was considered as statistically significant.

Results

Behavioral Observations in Rats

The seizures of NGF group and FPS-ZM1 group were decreased and the seizure duration was shortened.

The expression level of HMGB1 protein in the hippocampus of the young rats at 3h,24h,72h of the each group.

The HMGB1 expression level in hippocampus at each point time of group B was significantly higher than that of group A ($P = 0.00$). There was no significant difference in HMGB1 expression among of each point time of groups B ($P > 0.05$). The level of HMGB1 expression at each point time of group C and D was lower than that in group B, but higher than that in group A, the difference was statistically significant ($P < 0.01$).The HMGB1 expression level at 3 h and 24 h of group D was lower than that of group C ($P < 0.05$), but not at 72 h ($P > 0.05$). (Figure 1)

The expression level of HMGB1 protein in serum of at 3h,24h,72h in each group

The HMGB1 expression level in serum at each point time of group B was significantly higher than that of group A($P = 0.00$); There was no significant difference in HMGB1 expression among of each points in groups B, C and D ($P > 0.05$).

The level of HMGB1 expression at each points of group C and D was lower than that of group B, but higher than that in group A, the difference was statistically significant ($P< 0.01$); The HMGB1 expression level at 3 h and 24 h of group D was lower than that of group C ($P < 0.05$), but not at 72 h ($P > 0.05$). (Table 1)

Discussion

Epilepsy is a disorder of brain function caused by abnormal discharges of the brain. The incidence of epilepsy in children is about 0.2% [1], and epilepsy causes some impairment of brain function. Since early childhood and adolescence are important stages for brain growth and development, childhood brain damage seriously reduces their future quality of life, so it is important to reduce seizures and to reduce brain damage. At present, the mechanism of brain injury caused by epilepsy is very complicated, including neuronal apoptosis, blood-brain barrier destruction, immune inflammatory reaction caused by oxidative stress injury, etc. There were many animal studies and clinical studies have shown that epilepsy induces activation of inflammatory media, that the inflammatory response of the nervous system aggravates the epilepsy [2], and that anti-inflammatory therapy with corticosteroid is useful in the treatment of refractory epilepsy proved the role of inflammation in epilepsy. The focus of current epileptic research is on how to reduce oxidative stress and cerevrate inflammation in epileptic children.

HMGB1 is a highly conservative and widely distributed nuclear protein in eukaryotic cells. Previous studies have shown that it played an important role in the development of tissue ischemia, immune inflammation, metabolic disturbances, neurodegenerative diseases, tumors, etc. [3-4]. It also causes excessive excitability of neuron, decreases the threshold of convulsion, and leads to the development of epilepsy [5-6]. HMGB1 is an endogenous danger signal molecule and is also a core molecule in the inflammatory pathway. It has inflammatory factors and is also an important pro-inflammatory cytokine in the inflammatory pathway. When cells are damaged or necrotic, it can be translocated to extracellular space. It can also be secreted by mononuclear macrophages, colloid cells and dendritic cells when they are stimulated by inflammatory factors ,such as interleukin-1（IL-1）, and then HMGB1 are combined with its receptor– TRL4,it initiates the signal pathway of NF-κB, NF-κB translocate into nucleus ,regulate the activity of relate genes （participate the inflammatory reactions and immune reactions. At the same time, it can regulate the release of IL-2 and other inflammatory factors, promote and enlarge the inflammatory reaction, cause tissue damage, degeneration and necrosis, and cause a series of pathological changes. HMGB1 is often absent or low expression in normal neural tissue but elevated in damaged neural tissue. It has been shown that HMGB1 expression is closely related to epilepsy. Both clinical and basic studies have shown that HMGB1 expression increased in epileptic animal and human being. In my study, HMGB1 expression in hippocampus and serum of epileptic rats was significantly higher than that of controls group [8-9]. HMGB1 expression in epileptic rats was not significantly different among of 3 h, 24 h and 72 h , These results indicated that the risk signal molecule HMGB1 increased after the neuron cells were damaged in epilepsy, which might lead to a series of inflammatory reactions. Moreover, HMGB1 is secreted to the extracellular and can pass through the blood-brain barrier, induce the increase of serum HMGB1 . Therefore, we can evaluate degree of brain injury after epilepsy by testing serum HMGB1. HMGB1 expression in hippocampus and serum of epileptic rats started to increase in the third hour; it did not decrease at 72h. There was no significant difference in HMGB1 expression at each point time .So HMGB1 is a very sensitive indicator of neuronal injury. It rapidly increased after seizure. and lasts for a long time in the serum, so it can be used as a marker for epileptic injury. The expression of HMGB1 at each time point in hippocampus and serum of NGF group and FPS-ZM1 group were significantly lower than that of epilepsy group ,so HMGB1 can also be a indicator of therapeutic effect.

NGF, as a common neuroprotective agent, is necessary for the development and growth of the nervous system. NGF can promote the repair of the myelin sheath. NGF can also promote the repair of the nerve fibers and reduce damage of brain . NGF is often used in the treatment of cerebral palsy, cerebral infarction and peripheral neuropathy .In our previous basic research, we found that NGF can reduce the apoptosis of neurons, reduce the proliferation of glial cells, and protect brain .There was a study about treating children refractory epilepsy with NGF have shown that NGF combined with antiepileptic medicines decrease seizure and improves quality of life ,and it were little side effects[13]. Lei's research about animal study have indicated that NGF reduced seizures of epilepsy rats through the p75R/C pathway [14], and was expected to become a new antiepileptic medicine. The result of my study was the same with the result of Lei's study, the seizures of NGF group were less than those of epilepsy group.The expression of HMGB1 in hippocampus and serum at each time point of NGF group was lower than that of epilepsy group , the difference was statistically significant, NGF could reduce epileptic seizure in epileptic rats, and reduced brain damage. Therefore, NGF is expected to be used in therapy of epilepsy and protect the brain function , improve the quality of life of children in the future.

FPS-ZM1 is a RAGE (receptor for advanced glycation end-products)Antagonist, it can block the downstream signal transduction pathway and inhibit the inflammatory reaction by inhibiting the activity of RAGE. WANG 's study about cellular studies have shown that FPS-ZM1 reduced HMGB1 expression by inhibiting RAGE [4]. In the resent basic study, FPS-ZM1 could be used as an anti-inflammatory drug to reduce inflammatory injury in human . Foreign studies have shown that FPS-ZM1 could be used to treat inflammatory bowel disease and nervous system inflammation caused by Zika virus [15-16]. SHEN's study has found that FPS-ZM1 can block its downstream signaling pathway by inhibiting RAGE activity, reducing the activation of glial cells, oxidative stress, inhibiting the inflammatory response of human body, and having neuroprotective effects [17]. At present, there are few reports about using FPS-ZM1to treat children epilepsy and protect brain injury . In my study, FPS-ZM1 decreased the seizure and the expression of HMGB1 in hippocampus and serum of epileptic rats were significantly decreased compared with epileptic group, suggested that FPS-ZM1 might reduce oxidative stress in epileptic rats by inhibiting RAGE expression, thereby reducing the seizure activity and neuronal inflammatory response, and reducing the brain damage in epileptic rats. Moreover, the expression of HMGB1 in hippocampus and serum of FPS-ZM1 group were significantly lower than that of NGF group at 3h and 24h. It is suggested that the anti-inflammatory effect of FPS-ZM1 is superior to that of NGF, and it is expected to be a novel anti-epileptic medicine as well as a brain protective medicine in epilepsy children .

In conclusion, HMGB1 was significantly increased in hippocampus and serum of epileptic rats; it started to increase in the third hours, HMGB1 could be used as a marker of brain injury of epilepsy children . NGF and FPS-ZM1 could decrease seizure and it is effective in repairing the brain . The protective effect of FPS-ZM1 was better than that of NGF.

Declarations

Statement of Ethics

This experiment was approved by the Animal Ethics Committee of Fujian Provincial Hospital(2018-014).

Consent for publication

Consent for publication was obtained from all participants.

Availability of supporting data

The data sets supporting the results of this article are included within the article and its additional files.

Competing interests

The authors have no conflicts of interest to declare.

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Author Contributions

CHEN Qiao-bin and CHEN Lang designed the study, analyzed all the data and wrote the first manuscript drafts, Li-Dongmei. ZHENG Xin. FANG-Qiong performed the tests , All authors reviewed the manuscript.

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Abbreviations

Nerve Growth Factor NGF

Enzyme-linked Immunosorbent Assay ELISA

Western-blot WB

Pentylenetertrazol PTZ

Tris Buffered Saline Tween TBST

Receptor for Adcanced Glycation End Products RAGE

High Mobility Group Box-1 HMGB1

Interleukin-1 IL

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Table

Due to technical limitations, the table is only available as a download in the supplementary files section.

Figures

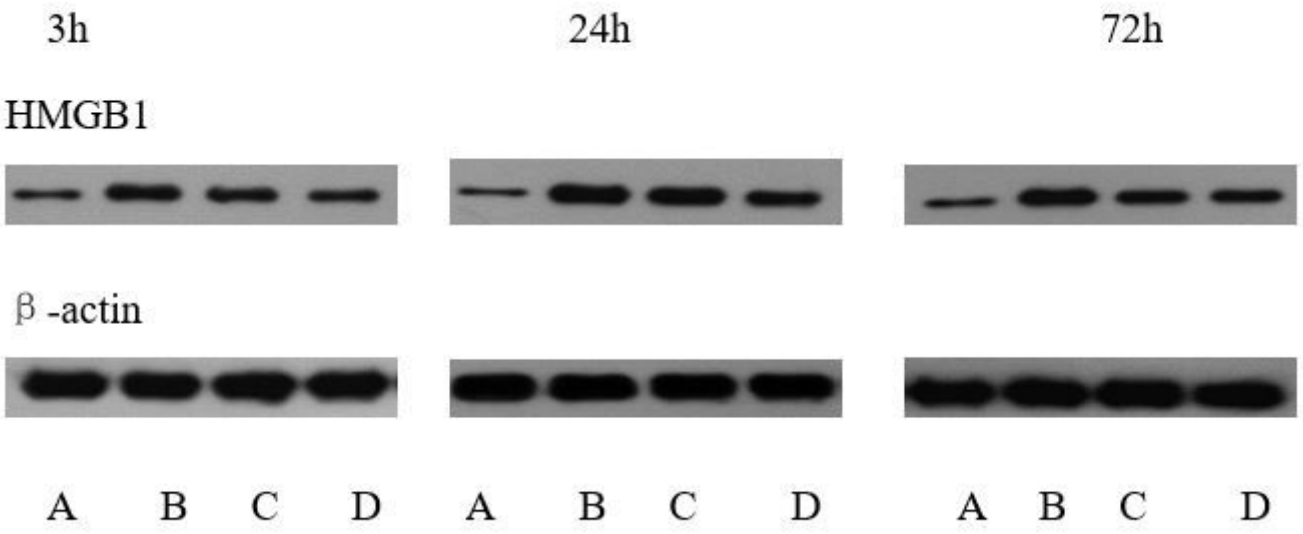

Figure 1

Western blot of HMGB1 protein expression in hippocampus

Supplementary Files

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