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Estradiol on Brain Pathological Changes in Ovariectomized Rats with Chronic Cerebral Ischemia

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Abstract

To investigate the protective effect of Ligustrazine on osteoporosis in diabetic rats. 28 healthy female SD rats, weighing 350 g-420 g, were provided by a medical college. Twenty-eight female rats were randomly divided into four groups: normal control group (group A), sham ovariectomized ischemia group (group B), ovariectomized ischemia group (Group C) and estradiol benzoate treatment group (Group D), with 7 rats in each group. Estradiol benzoate was added 1 week after ovariectomy. After 3 weeks, the forebrain ischemia model was established by 2VO except the normal control group, and the experiment was continued for 5 weeks. The density of capillaries and capillaries of the model rats were observed by immunohistochemical staining, and the density of capillaries and capillaries of the model rats were observed. Finally, the data were statistically analyzed. Compared with the normal control group, the density of neurons and capillaries in ischemic group was significantly decreased. Compared with OVX + EB + 2VO group and shamovx + 2VO group, the density of neurons and capillaries in OVX + 2VO group was significantly lower than that in OVX + EB + 2VO group ($P < 0.05$). Compared with the normal control group, the density of hippocampal CA1 neurons in ischemic group was decreased. At the beginning of the experiment, there was no significant difference in the latency of each group. At the end of the experiment, except for the normal control group, the latency of other groups was significantly longer than that at the beginning of the experiment. The mortality of shamovx + 2VO group was 0.3, that of OVX + EB + 2VO group was 0.6, and that of OVX + 2VO group was 0.367. The mortality of OVX + 2VO group was higher than that of sham OVX + 2VO group and OVX + EB + 2VO group, but the difference was not statistically significant ($P > 0.05$). Estrogen has protective effect on the behavior changes of rats with cerebral ischemia before ovariectomy, and has beneficial effect on pathological changes of cerebral ischemia injury. Estradiol has a certain protective effect on chronic cerebral ischemia.

Keywords: Chronic Cerebral Ischemia, Ovariectomized Rats, Estradiol Research, Pathological Changes

1.Introduction

The energy reserve of brain tissue is very limited [1-2]. Once the blood supply is reduced or stopped, energy metabolism disorder and failure will occur rapidly, leading to nerve dysfunction and necrosis or apoptosis of ischemic region [3-4]. Cerebral ischemia can be divided into acute ischemia and chronic ischemia [5]. In recent years, chronic cerebral ischemia has been proved to be a common pathological process in the development of vascular dementia, Binswanger disease and other diseases [6-8]. Permanent ligation of bilateral common carotid arteries (2VO) was used in chronic cerebral ischemia model.

Cerebral blood flow decreased significantly after bilateral common carotid artery ligation [10]. However, due to the good development and compensation of the cerebral artery ring, the blood supply of no brain area was completely terminated

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after ligation, and the rat brain was in the state of low perfusion [11-12]. Then a series of histopathological, energy and behavioral changes were induced [13-15]. Estrogen can regulate not only reproductive related areas of the brain, but also non reproductive areas related to learning and memory, such as cerebral cortex and hippocampus [16-17]. Estrogen is a kind of sterol sex hormone of seedling [18].

Estrogen plays an important role in promoting the development and maturity of sexual organs, maintaining secondary sexual characteristics, regulating energy metabolism, and changing fat distribution [19-20]. Permanent bilateral common carotid artery ligation is a good model for the study of forebrain ischemia and cognitive function [21-22]. It can simulate vascular dementia caused by atherosclerosis and lumen stenosis [23]. After several months of operation, the cerebral blood flow decreased significantly. The Willis Circle of rats developed well and compensated well, and the cerebral perfusion was not completely stopped [24]. The permanent ligation of bilateral common carotid arteries is simple and easy to operate, with high success rate of modeling, and can maintain the basic function of life center, which is suitable for long-term research. It is a good learning forebrain ischemia model and cognitive dysfunction [25].

2. Theoretical Basis

2.1. Chronic Cerebral Ischemia

Replacement therapy can reduce the relative risk of stroke and stroke related mortality. In the process of chronic cerebral ischemia, the cerebral blood flow decreased sharply. As a result of ischemia and hypoxia, some small blood vessels in the brain shrink and the lumen is blocked, which leads to serious blood supply insufficiency, energy metabolism disorder and neuron death. At the same time, due to the slow and continuous cerebral hypoperfusion, its intensity is not as strong as acute cerebral ischemia caused by acute vascular occlusion. We can observe that there are two pathological changes in the hippocampal parietal cortex and hippocampal CAI area: one is the formation of infarction, which may be caused by occlusion of small arteries after ischemia and hypoxia; the other is the death of scattered nerve components. This may be related to neuronal death caused by slow and persistent cerebral ischemia in rats. In these two pathomorphological changes, we speculate that necrosis and apoptosis may occur, leading to neuronal death. After chronic cerebral ischemia, the outer membrane cells of capillaries degenerate, the basement membrane thickens, the lumen narrows and distorts; the density of capillaries decreases and the number of atrophic vessels increases. It can be seen that with the aggravation of capillary ischemia injury, the local regional blood supply may be further reduced, leading to nerve repair dysfunction after injury. Therefore, the integrity of capillaries plays an important role in maintaining brain function during ischemia. As far as the nervous system is concerned, it does not act as a neurotransmitter or a second messenger, and plays an important role in the transmission of neural information, the formation of synaptic plasticity, the process of learning and memory, the development of nervous system and the regulation of cerebral blood flow. There were local differences in the distribution of NOS activity in the brain, with the highest activity in cortex, striatum, hippocampus and hypothalamus. Nitric oxide is a key transmitter of normal learning and memory. NNOS and eNOS are expressed in hippocampal circuits, which are related to cognitive function.

In addition, eNOS may be related to synaptic plasticity of hippocampal pyramidal cells. No acts as a retrograde messenger in the long-term potentiation (LTP) of hippocampus. No and LTP may be the molecular and neural basis of learning and memory. It is of great significance to maintain normal brain function and prevent dementia to maintain the appropriate concentration of no in brain. After cerebral ischemia, the synthesis and release of no increased, and it reacted with superoxide anion 02 rapidly to form small peroxide group (onoo-1), which was further protonated into peroxymethylene (on00h) and decomposed into oh - and N02 in acidic environment. Oh - is an important oxygen free radical in brain injury induced by No. the direct oxidative toxicity of ONOO - is much greater than that of OH - 1. It is not only a strong oxidant, but also has a high selectivity to the reaction. Peroxides can directly oxidize lipids, DNA and protein bases, zinc, etc. These products can cause serious cell damage. No also leads to DNA damage and inhibition of RNA reductase, which is a rate limiting enzyme of DNA synthesis. No and its products ONOO and oh can also cause DNA oxidation, destroy DNA structure and further damage DNA. It is the result of many factors, such as the decrease of blood flow in ischemic region, the increase of excitatory amino acid release, the influx of calcium ions and the disorder of cellular energy metabolism. In the process of neuroprotection and repair after chronic cerebral ischemia, the above two mechanisms must play their respective important roles and interact with

each other. As chronic ischemic cerebral hypoperfusion is the most fundamental cause of brain injury, estrogen may regulate cerebral blood flow through volume dependent mechanism. Reduce damage. Estrogen can improve the blood supply of cerebral ischemia area. In a sense, it can also regulate cerebral blood flow.

2.2. Estrogen

Estrogen is a steroid hormone. There are three estrogens in human body: estradiol, estradiol and its metabolite estradiol. Estradiol is the main component of estrogen, and its biological efficiency is the strongest. The biological activity of estradiol is only 1 / 10 of that of estradiol, while the biological activity of estradiol is low and has only partial effect. The main organs that synthesize estrogen are ovary, adrenal gland and placenta. Bcl-2 family is an important regulator of apoptosis. Estradiol can increase the expression of glial derived TGF - Akt cells, further confirming its potential signaling mechanism to PI3K / Akt cells. We found that the expression of TGF mRNA in astrocytes was up-regulated after cerebral ischemia, which was parallel to the survival of ischemic neurons. Estrogen, IGF-1 and its binding protein-2 are involved in the pathophysiological process of synaptic plasticity and nerve injury repair. Further immunohistochemical localization analysis confirmed that IGF-1 and IGF-2 binding protein-2 originated from a special astrocyte elongation cell. Activated astrocytes are closely related to cerebral ischemic tolerance. ApoE is mainly synthesized by astrocytes in the central nervous system. ApoE is involved in the down regulation of inflammatory response in central nervous system. It can inhibit the secretion of tumor necrosis factor-A by glial cells and alleviate ischemic injury. ApoE can also promote neuronal repair by sending lipids to ischemic neurons.

Estrogen changes intracellular Ca2 + concentration through the phospholipase C signal transduction pathway mediated by estrogen receptor on cell membrane, thus affecting various functions of astrocytes. At the same time, arachidonic acid rapidly mobilizes activated protein kinases, such as calcium / calmodulin dependent kinase and phospholipid dependent protein kinase caca2 +, which can affect the proliferation and differentiation of astrocytes, the reconstruction of bone scaffold, gene expression, and finally the proliferation and differentiation of astrocytes. Astrocytes and microglia are important cells involved in immune response and neuronal degeneration in cerebral cortex. Estrogen and selective estrogen receptor modulator raloxifene can affect the expression of immune neurotransmitters (such as cytokines, complement, chemokines and other molecules) in astrocytes and microglia, in neuroinflammatory response and neurodegenerative diseases, and affect glial mediated inflammatory pathways to play a protective role. Estrogen is involved in the synthesis of heme oxygenase and heat shock protein, which may have neuroprotective effect. Hypoxia is the most sensitive to VEGF expression. The pathological changes caused by cerebral infarction / reperfusion are the changes after cerebral hypoxia ischemia. Therefore, it is bound to increase the expression of VEGF. This study confirmed that cerebral infarction / reperfusion injury can increase the expression of VEGF protein and mRNA, which is consistent with the previous results. When a certain hormone or drug is added, the expression of VEGF will be affected. As the expression of VEGF has protective effect on injured brain tissue, when some factors lead to high expression of VEGF, it can produce brain protection. As an important brain protective factor, estrogen can regulate the proliferation, migration and survival of vascular endothelial cells. It can increase the density of capillaries in infarct area, mediate angiogenesis and protect brain. Estrogen can enhance the regulation of blood vessels and promote the proliferation, migration and survival of vascular endothelial cells. Estrogen binds to the corresponding receptor protein in vascular endothelial cells and smooth muscle cells to form complex, and recognizes that estrogen effector elements in the DNA sequence of its target gene form an estrogen receptor estrogen response element, which regulates the biosynthesis of cycloprostaglandins, does not, and modulates the release, leading to vasodilation and increasing cerebral blood flow in ischemia. High dose of 17 estradiol can maintain the integrity of the cerebral blood flow barrier and help to maintain the cerebral blood flow.

2.3. Ovariectomy Test

There is a certain relationship between estrogen level and ischemic cerebrovascular disease. The incidence rate of stroke in premenopausal women is lower than that in men of the same age, but the incidence rate of stroke increases significantly in postmenopausal women. Estrogen can inhibit apoptosis, which may be achieved through the genetic effect of estrogen. It is known that Bcl-2 family is an important regulator of apoptosis, including Bcl-2, BDXL and promoters Bax, bax-5 and so on. The relative level of these two factors affects

the sensitivity of cells to apoptosis. Estrogen binds to the estrogen response elements (eres) in the enhancer region of Bcl-2 and Bcl XL genes through classical gene pathway, and regulates the expression of Bel-2 and belxl. Dubai aluminum, etc. It was found that in MCAO model, daily increase of ER in cerebral cortex paralleled with BD-2 and estrogen treatment of cerebral ischemia showed that estrogen combined with ER may increase the expression of BD-2 in ischemic cortex, and estrogen can increase the expression of Bcl-2 in ischemic penumbra. Estrogen also increased the expression of bol2 in NT-2 cells and arcuate nucleus neurons. Similarly, estrogen can also increase the level of bclxmrna in PC12 cells.

Estrogen may also play a neuroprotective role by inhibiting the neurotoxicity of excitatory amino acid receptors after ischemia. Estrogen can also couple with G protein on cell membrane through non genetic pathway and regulate Ca2 + channel through L-type voltage, thus reducing Ca2 + influx and preventing intracellular Ca2 + overload. In addition, estrogen may play an antioxidant role and other mechanisms to reduce neuronal apoptosis and loss. After cerebral ischemia, the synthesis and release of no were affected, and the content of NOS in brain was much lower than the normal level. Estrogen supplementation can reduce this phenomenon. This may be due to estrogen can directly promote the expression of NOS, resulting in the increase of NO synthesis. The energy metabolism in the brain after ischemia is also an important reason for the expression of Nos. estrogen can effectively improve the level of energy metabolism and maintain the stability of microcirculation. Estrogen can increase the expression of GLUT-1, maintain brain energy supply, and play a protective role in cerebral ischemia. The number of surviving neurons in ischemic region of rats transfected with GLUT-1 gene before MCAO was significantly higher than that of rats transfected with GLUT-1 gene. In the ischemic penumbra, the expression of GLUT-1 in estrogen pretreated rats was significantly increased. The ability of GLUT-1 to transport blood glucose through blood-brain barrier may be the rate limiting step of glucose to energy under cerebral ischemia and hypoxia. Increasing the expression of GLUT-1 can enhance the ability of brain to absorb energy, improve the survival rate of capillary endothelial cells, maintain the stability of blood-brain barrier, and reduce the possibility of brain energy failure to a certain extent. From this point of view, estrogen may play a role in promoting energy metabolism compensation and maintaining cell function after ischemia.

3. Experiment

3.1. Research Object

Twenty-eight healthy female SD rats, weighing 350g-420g, were provided by a medical college, with natural circadian rhythm, drinking and feeding freely, weighing once a week.

3.2. Animal Grouping

Methods: Twenty eight female rats were randomly divided into four groups: A: normal control group, n = 7; B: sham ovariectomy + 2VO, n = 7; C: ovariectomized ischemia group (OVX + 2VO), n = 7; D: estradiol benzoate treatment group (OVX + EB + 2VO), n = 7, and began to supplement estradiol benzoate (EB) 1 week after ovariectomy. After 3 weeks, the forebrain ischemia model was established by 2VO except the normal control group, and the experiment was continued for 5 weeks. In estrogen group, EB was injected intramuscularly one week after operation. The formula of dose conversion was: rat dose (mg / kg) = w × human oral dose (mg / kg). The dose of the population is 1mg / 60kg / 3D, W is the conversion coefficient, the experimental value is 6.25, so the dose is about 0.1mg/kg. Weight was measured once a week to adjust the dose. The other experimental groups were given 0.04 ml normal saline every 3 days.

3.3. Experimental Plan

Preparation of OVX model: rats were fasted for 12 hours before operation. 10% chloral hydrate (300mg / kg) was injected intraperitoneally. Rats were fixed in supine position. The abdominal skin was cut and disinfected. The skin was cut about 2 cm along the midline of the abdomen. The oviduct can be seen from the ventral side by pushing the bowel tube. Mulberry like red solid tissue can be seen at the end of fallopian tube. The remnant end was ligated with silk thread, and the other side underwent oophorectomy. After the operation, the abdominal cavity was closed and the incision was sprayed with penicillin powder. In the sham ovariectomized group, only the same amount of adipose tissue around the ovary was removed.

Chronic cerebral ischemia model (2VO): fasting 8-12h before operation, supine fixation under ether anesthesia. Cut the neck skin and separate the quadratus muscle and hyoid muscle of shoulder swelling. The distal and proximal ends of bilateral common carotid arteries were ligated with 4-0 silk thread. Bilateral common carotid arteries were

dissected. Routine disinfection, wound suture. Water maze test: Morris water maze was used for screening before operation, and memory function of rats was tested before death. Two times a day, rats were put into water from four water entry points facing the pool wall, and their incubation periods were recorded. If the platform was not found within 2 minutes, the rats were placed on the platform for 30 seconds. The latency was recorded as a maximum of 120 seconds. Every time the animals were put into the water, the experimenters withdrew immediately to avoid human interference. After each training, the mice were dried in the sun and kept warm in their cages. The death rate of rats after ischemia was calculated: the death rats were counted after bilateral common carotid artery ligation, and the injury related death such as anesthesia accident, compression of common carotid sinus, tracheal asphyxia and massive hemorrhage were excluded. Mortality = dead rats / total number of rats in each group × 100%.

Preparation of pathological specimens: after the experiment, rats in each group were anesthetized with 10% chloral hydrate (300mg / kg). After the chest was opened to expose the heart, the right atrial appendage was cut with small scissors, and the left ventricle was intubated with normal saline. Then slowly inject 10% formaldehyde for fixation, and then decapitate. The intact rat brain was fixed with 4% paraformaldehyde, dehydrated and transparent, and embedded in paraffin at low temperature. He staining, LFB + tar violet staining and CD31 immunohistochemical staining were performed in the corresponding frontal lobe and hippocampus.

He staining method: the cut tissue sections were put into the section basket for dewaxing; staining: the section basket was placed in the slice basket, stained for 10-15 minutes, the remaining staining solution was washed with tap water, stained with hydrochloric acid and alcohol for 30 minutes, and then stained with eosin for 5 minutes, and then dehydrated. SP immunohistochemical staining: distilled water cleaning, hematoxylin staining, dehydration, transparent, sealed, observed under the microscope.

The morphological structure and distribution of neurons and myelin sheath were observed under microscope. He and CD1 immunohistochemical staining were used to observe the morphology and distribution of capillaries. At 10 × 40 times field of vision, 5 non overlapping visual fields were randomly selected from the superficial and deep epithelial layers, each sample was in the same position, and the pyramidal cell layer of hippocampal CA1 area in the visual field. The nerve cells and capillaries observed in the field of vision were recorded and their average values were calculated. The morphological changes of neurons and capillaries were compared and analyzed.

Statistical processing: the mean value ± standard deviation (x ± s) expressed by the measured data, the data of multiple groups of mean variance analysis were compared, and the comparison data rate calculated by multiple comparison method was tested by x2 test, and the difference was statistically significant ($P < 0.05$).

4. Experimental Results

4.1. Quantitative Analysis under Light Microscope

(1) Comparison of capillary density

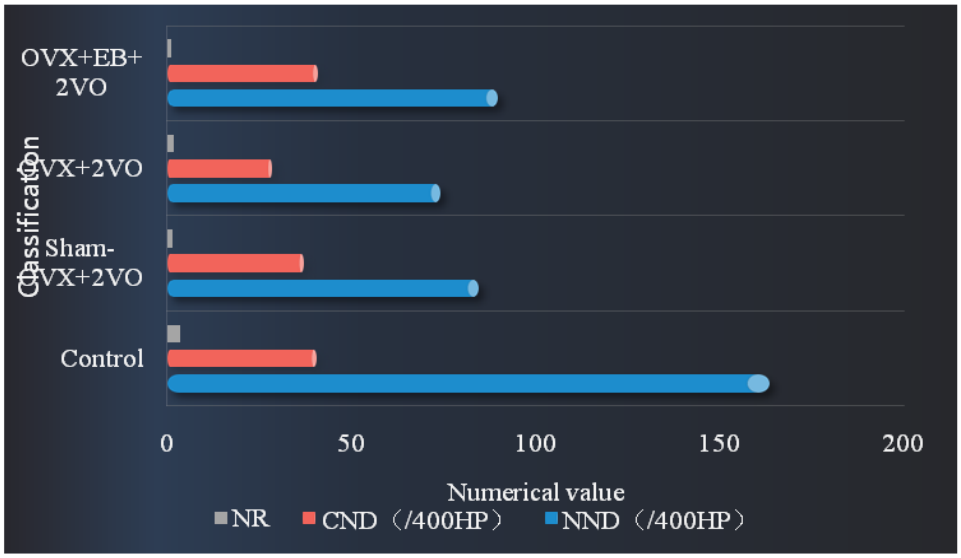

Figure 1. **Comparison of capillary density**

Table 1. **Comparison of capillary density**

Group	NND(/400HP)	CND(/400HP)	NR
Control	163.50±6.42	40.60±8.26	3.34±0.63
Sham-OVX+2VO	84.56±6.44	37.20±3.51	1.28±0.32
OVX+2VO	74.30±2.24	28.45±5.11	1.85±0.48
OVX+EB+2VO	89.84±6.98	41.12±2.13	1.15±0.14

According to the statistical analysis of data, as shown in Figure 1 and table 1, compared with the normal control group, the density of neurons and capillaries in the ischemic group was significantly reduced. Compared with OVX + EB + 2VO group and shamovx + 2VO group, the neuron density and capillary density of OVX + 2VO group were significantly decreased ($P < 0.05$), but there was no significant difference between OVX + EB + 2VO group and shamovx + 2VO group.

(2) Comparison of neuron density

*Table 2.***Comparison of neuron density**

Group	N	NND(/400HP)
Control	4	38.933±48.56
Sham-OVX+2VO	8	30.458±33.18
OVX+2VO	5	24.075±31.13
OVX+EB+2VO	7	32.333±43.53

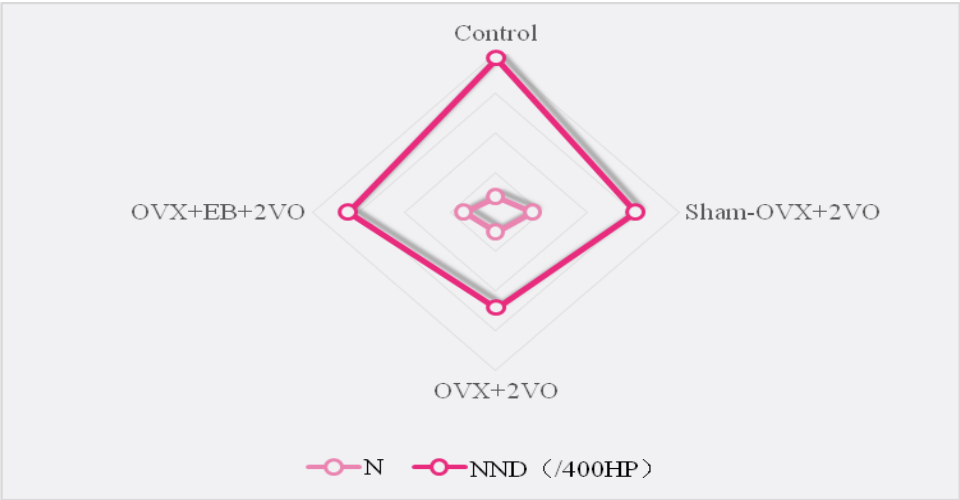

Figure 2. **Comparison of neuron density**

According to the statistical analysis of data, as shown in Fig. 2 and table 2, the density of neurons in hippocampal CA1 area of ischemic group was lower than that of normal control group. Compared with OVX + EB + 2VO group and shamovx + 2VO group, the density of neurons in OVX + 2VO group was significantly decreased ($P < 0.05$), but there was no significant difference between OVX + EB + 2VO group and shamovx + 2VO group.

4.2. Water Maze Test Results

(1) Latency comparison

Table 3. **Latency comparison**

Group	The beginning of the experiment	The end of the experiment
Control	10.09±3.43	10.65±3.29
Sham-OVX+2VO	10.17±5.58	23.32±2.99
OVX+2VO	10.16±6.19	32.43±4.90
OVX+EB+2VO	10.09±3.43	20.59±5.89

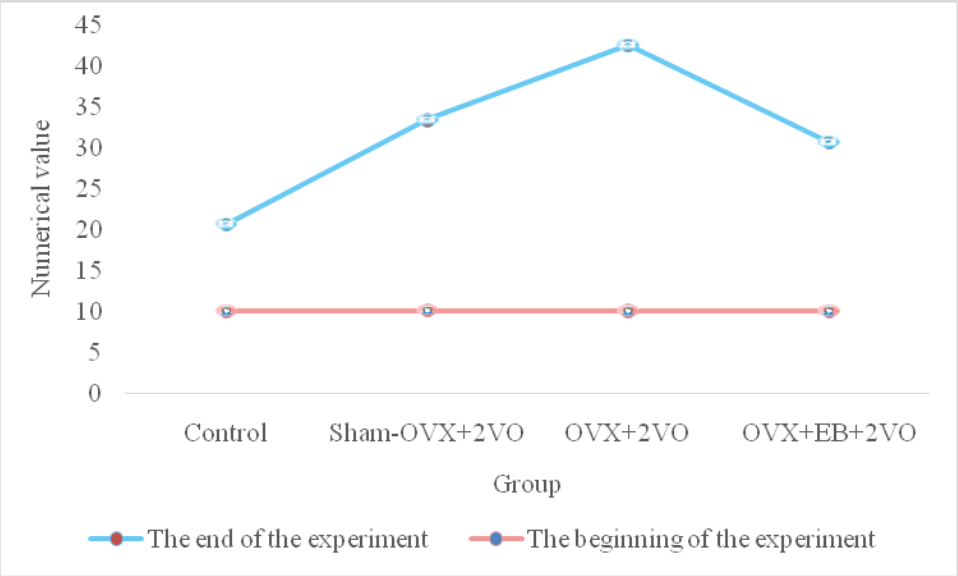

Figure 3. **Latency comparison**

According to the statistical analysis of the data, as shown in Fig. 3 and table 3, there was no significant difference in the latency of each group at the beginning of the experiment. At the end of the experiment, except for the normal control group, the significant delay time of other groups was more obvious than that of OVX + EB + 2VO and false OVX + 2VO at the beginning of the experiment ($P < 0.05$).

(2) Death situation

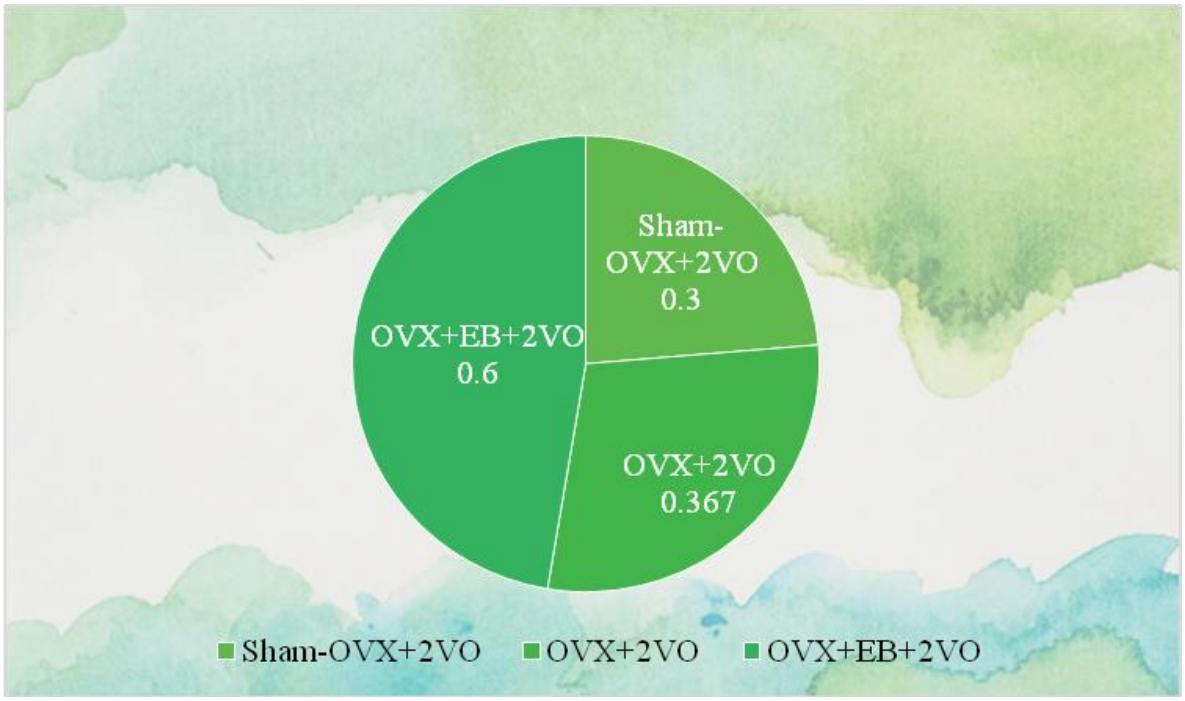

Figure 4. **Death situation**

According to the statistical analysis of data, as shown in Figure 4, rats died within 24 hours after bilateral common carotid artery ligation. The rats died of acute ischemia such as dryness, jumping and convulsion. There was no death after 24 hours. The mortality of shamovx + 2VO group was 0.3, that of OVX + EB + 2VO group was 0.6, and that of OVX + 2VO group was 0.367. The mortality of OVX + 2VO group was higher than that of sham OVX + 2VO group and OVX + EB + 2VO group, but the difference was not statistically significant ($P > 0.05$).

5. Analysis and Discussion

5.1. Analysis of Experimental Results

Glutamate, the main excitatory neurotransmitter in the brain, produces a decrease in glutathione (GSH) levels. By activating receptors, neurons also produce a large number of intracellular oxygen free radicals, including superoxide anion and hydrogen peroxide, which destroy cell membrane and ATPase activity, lead to membrane depolarization and calcium influx, leading to neuronal death and / or injury. Studies have shown that estradiol can reduce the free radical damage induced by cerebral ischemia-reperfusion, and reduce the excitability of insular cortical neurons caused by GABA release after permanent focal cerebral ischemia. We found that G1 can activate ERK1 / 2 signaling pathway and selectively down regulate the expression of NMDA receptor glun2b. These studies confirmed the neuroprotective effect of GPR30 on cerebral ischemia. However, GPR30 is also highly expressed in female reproductive organs such as breast, ovary and uterus, and is involved in the proliferation of these tissues. Therefore, long-term systemic activation of GPR30 can also lead to breast cancer, endometrial cancer and other tumors. Although these studies explore many goals and mechanisms for the prevention and treatment of stroke, such as the application of antioxidants, calcium blockers, glutamate receptor blockers and neurotrophic factors, researchers usually focus on direct neuroprotection, The potential neuroprotective effects of other neurons (astrocytes, microglia, oligodendrocytes) may also be critical. In recent years, astrocytes have gradually become the target of neuroprotection. Astrocytes are the most abundant cell types in the nervous system of higher mammals, which play a key role in the physiological activities and pathological damage of the central nervous system. Ischemic stroke is a kind of oxidative stress, excitotoxicity, inflammatory reaction, apoptosis and death caused by cerebral blood flow interruption. Compared with astrocytes, neurons are more sensitive to ischemia and hypoxia, with less endogenous antioxidants, and are prone to glutamate excitotoxicity. Although astrocytes are more resistant to cerebral ischemia than neurons, the neuroprotective effect of astrocytes after cerebral ischemia will be greatly reduced before apoptosis and necrosis, and even play the role of nerve injury. Damaged astrocytes aggravate neuronal death.

Estrogen is a steroid hormone. There are three estrogens in human body: estradiol, estradiol and its metabolite estradiol. Estradiol is the main component of estrogen, and its biological efficiency is the strongest. The biological activity of estradiol is only 1 / 10 of that of estradiol, while the biological activity of estradiol is low and has only partial effect. The changes of estrogen level in ovariectomized natural aging rats were similar to those in postmenopausal women and ad multi system aging. At the same time, cholinergic system of basal forebrain, synaptic function of hippocampus and learning and memory function decreased. In OVX + 2VO group, endothelial cells contracted, tight junctions separated, intercellular spaces widened, and even endothelial cells fell off. These changes lead to increased vascular permeability and cause and aggravate brain edema. At the same time, due to the compression of brain edema, the shape of microvessels has circuitous, spherical and linear changes, resulting in blood stasis, forming microthrombosis, reducing the amount of cerebral perfusion and aggravating cerebral ischemia injury. The number of capillaries in OVX + EB + 2VO group and shamovx + 2VO group was significantly higher than that in OVX + 2VO group, suggesting that estrogen has a good protective effect on vascular endothelial cells and promotes vascular proliferation. VEGF is also a survival factor for newly formed endothelial cells. Without VEGF, endothelial cells die quickly.

Cerebral ischemia and hypoxia, as a signal, activate the vascular endothelial growth factor / receptor system, promote the high expression of VEGF in brain tissue ischemia and hypoxia, promote the proliferation of vascular endothelial cells, produce a large number of new blood vessels, improve the blood supply of injured brain tissue and reduce brain injury. After bilateral common carotid artery ligation, the expression of VEGF was highest at 1 week after cerebral ischemia, decreased at 2 weeks, continued to decrease at 4 weeks, and still had a little expression until 8 weeks. The expression of VEGF was mainly concentrated in cerebral cortex and hippocampus. Compared with sham OVX + 2VO group, the number of capillaries decreased after ovariectomy. After estrogen supplement, the number of capillaries increased and the damage degree of endothelial cells decreased. Estrogen plays an important role in vascular protection by regulating VEGF. On the one hand, estrogen can protect vascular endothelial cells, on the other hand, it can promote the proliferation of capillary after ischemia. This not only promotes the increase of the number of blood vessels, but also protects the function of vascular endothelial cells. In OVX + 2VO group, a large number of ischemic neurons appeared in frontal

cortex. In OVX + EB + 2VO group and sham OVX + 2VO group, the neurons showed mild ischemic changes, a few neurons degenerated and necrotic, LFB staining showed no obvious demyelination. The density of neurons in OVX + EB + 2VO group and sham OVX + 2VO group was higher than that in OVX + 2VO group ($P < 0.05$). Compared with sham OVX + 2VO group and normal control group, the number of neurons in hippocampal CA1 area in OVX + 2VO group was decreased and disordered ($P < 0.05$).

5.2. Discussion

In ischemic tissue, neovascularization is the basis of improving collateral circulation dependent blood flow. Proliferating blood vessels can improve the blood supply of neurons and protect neurons after ischemia. In addition, the vascular protective effect of estrogen is also related to the following factors: estrogen can directly act on vascular smooth muscle and endothelial cells to dilate blood vessels and increase cerebral blood flow. Estradiol can increase the activity of nitric oxide synthase (NOS) in vascular endothelial cells and neurons, so as to increase the local concentration of no, relax the blood vessels and increase the cerebral blood flow; estrogen can reduce the structural damage of cerebral microvessels, reduce the vascular permeability and improve the cerebral microcirculation; estrogen can regulate the metabolism and deposition of cholesterol, and reduce the serum low density lipoprotein, Molecular biology technology has detected estrogen receptor in cardiovascular system. Many of the vascular protective effects of estrogen are mediated by estrogen receptors in the vascular wall, such as inhibiting the proliferation of vascular smooth muscle cells, promoting the release of no from vascular endothelial cells, and repairing endothelial injury. Therefore, the quality and quantity of Er play an important role in vascular protection. The hippocampus is sensitive to a variety of stimuli, but also contains rich estrogen receptors. The formation of hippocampus is an important part of the limbic system, which plays an important role in learning and memory.Estrogen affects the central cholinergic, glutamatergic and monoaminergic nervous system functions through estrogen receptor, participates in the regulation process of learning and memory, and can reduce the damage of hippocampal memory function after ischemia.

Inflammatory reaction plays a beneficial and harmful role in cerebral ischemia, which depends on the time and severity of inflammatory reaction. Within a few minutes after cerebral ischemia, neurons and glial cells in the damaged core and penumbra produced a large amount of proinflammatory mediators, cytokines and reactive oxygen species. These substances activate astrocytes. In addition to inflammatory factors, reactive astrocytes can also produce a large number of chemokines, which can attract immune cells by up regulating vascular endothelial cell adhesion molecules, causing immune cascade reaction and aggravating the inflammatory injury of cerebral ischemia. Although little attention has been paid to the potential benefits of post-stroke inflammatory response, there is indirect evidence that some specific inflammatory reactions have neuroprotective and neural regeneration effects. In addition to defending against the invasion of pathogens, inflammation can also help remove necrotic tissue, promote angiogenesis, tissue reconstruction and regeneration. Especially in the process of wound healing, if the inflammatory reaction is inhibited, the tissue will lack of resistance.

On the one hand, estrogen can reduce the damaged neurons, on the other hand, it can also protect the function of surviving neurons. Estrogen is related to the degree of chronic cerebral ischemia injury, which has beneficial effect on the pathological changes of chronic cerebral ischemia injury and provides pathological basis for the recovery of injured nerve cells. Estrogen can promote the regeneration of capillaries after ischemia and enhance the protection of neurons after ischemia, which lays a foundation for further clinical research on prevention and treatment of ischemic cerebrovascular disease by estrogen. However, estrogen cannot completely prevent the pathological damage and behavior changes after ischemia, and cannot reduce the mortality of acute ischemic phase.

6. Conclusion

Ovariectomy combined with bilateral common carotid artery ligation is a good animal model to simulate chronic cerebral ischemia in postmenopausal women; the degree of cerebral ischemia injury is related to the level of estrogen; estrogen has protective effect on the behavior changes of ovariectomized cerebral ischemia rats; estrogen can promote the pathological changes of cerebral ischemia injury to a certain extent. However, estrogen could not reduce the mortality of ovariectomized rats.

References

[1] Scholz-Ahrens, K. E., Adolphi, B., Rochat, F.,

Barclay, D. V., De Vrese, M., Açil, Y., & Schrezenmeir, J. (2016) "Effects of Probiotics, Prebiotics, and Synbiotics on Mineral Metabolism in Ovariectomized Rats-Impact of Bacterial Mass, Intestinal Absorptive Area and Reduction of Bone Turn-Over", *NFS Journal*, 3(C), pp.41-50.

[2] Ramalho-Ferreira, G., Faverani, L. P., Momesso, G. A. C., Eloá, R. L., & Okamoto, R. (2016) "Effect of Antiresorptive Drugs in the Alveolar Bone Healing. AHistometric and Immunohistochemical Study in Ovariectomized Rats", *Clinical Oral Investigations*, 21(5), pp.1-10.

[3] Wang, Q., Liu, Z. W., Wang, Y. N., Pan, Q. Y., Feng, Q. J., Huang, Q.H., & Chen, W. F. (2016) "Quantitative Ultrasound Assessment of Cartilage Degeneration in Ovariectomized Rats with Low Estrogen Levels", *Ultrasound in Medicine & Biology*, 42(1), pp.290.

[4] Zhang, Y., Wang, Z., Ding, L., Damaolar, A., Li, Z., & Qiu, Y., Yin, Z. (2016) "Lentivirus-Taz Administration Alleviates Osteoporotic Phenotypes in the Femoral Neck of Ovariectomized Rats", *Cellular Physiology & Biochemistry International Journal of Experimental Cellular Physiology Biochemistry & Pharmacology*, 38(1), pp.283.

[5] Nie, M., Zhang, Q., Zhang, G., Chen, J., & Dong, Q. (2017) "Synthesis of Resveratrol Loaded Mpeg-PlaNanomicelles for the Prevention of Osteoporosis in Ovariectomized Rats", *Latin American Journal of Pharmacy*, 36(10), pp.2112-2119.

[6] Li, S. W., Niu, G. D., Dong, N. X., Wang, X. D., Liu, Z. J., Song, C. L. & Leng, H. J. (2017) "Osteoporosis Affects Both Post-Yield Microdamage Accumulation and Plasticity Degradation in Vertebra of Ovariectomized Rats", *ActaMechanicaSinica*, 33(002), pp.267-273.

[7] Zhang, J. M., Li, J., Liu, E. W., Wang, H., Fan, G. W., & Wang, Y. F. (2016) "Danshen Enhanced the Estrogenic Effects of Qing e Formula in Ovariectomized Rats", *Bmc Complementary & Alternative Medicine*, 16(1), pp.181.

[8] Zhou, R. P., Lin, S. J., Wan, W. B., Zuo, H. L., & Luo, J. (2016) "Chlorogenic Acid Prevents Osteoporosis by Shp2/Pi3k/Akt Pathway in Ovariectomized Rats", *Plos One*, 11(12), pp.e0166751.

[9] Habibi, P., Alihemmati, A., Nasirzadeh, M., Yousefi, H., Habibi, M., & Ahmadiasl, N. (2016) "Involvement of Microrna-133 and -29 in Cardiac Disturbances in Diabetic Ovariectomized Rats", *Iranian Journal of Basic MedicalEnces*, 19(11), pp.1177.

[10] Zhou, H., Hou, Y., Zhu, Z., Xiao, W., & Chen, W. (2016) "Effects of Low-Intensity Pulsed Ultrasound on Implant Osseointegration in Ovariectomized Rats", *Journal of Ultrasound in Medicine: Official Journal of the American Institute of Ultrasound in Medicine*, 35(4), pp.747.

[11] Li, C., Sun, H., Arrick, D. M., & Mayhan, W. G. (2016) "Chronic Nicotine Exposure Exacerbates Transient Focal Cerebral Ischemia-Induced Brain Injury", *Journal of Applied Physiology*, 120(3), pp.328-333.

[12] Antipenko, E. A., Derugina, A. V., & Gustov, A. V. (2016) "The System Stress-Limiting Action of Mexidol in Chronic Cerebral Ischemia", *Zhurnal Nevrologiii Psikhiatrii Imeni s. s. Korsakova*, 116(4), pp.28.

[13] Mao, H. F., Xie, J., Chen, J. Q., Tang, C. F., Chen, W., Zhou, B. C., Chen, R., Qu, H. L., & Wu, C. Z. (2017) "Aerobic Exercise Combined with Huwentoxin-i Mitigates Chronic Cerebral Ischemia Injury", *Neural Regeneration Research*, 12(004), pp.596-602.

[14] Anisimova, A. V., Gunchenko, A. S., Ikonnikova, A. Y., Galkin, S. S., & Nasedkina, T. V. (2019) "A Clinical and Genetic Analysis of Risk Factors for the Development of Acute and Chronic Cerebral Ischemia", *Zhurnal Nevrologiii PsikhiatriiImeni S. S. Korsakova / Ministerstvo Zdravookhraneniiai Meditsinskoi Promyshlennosti Rossiiskoi Federatsii, Vserossiiskoe Obshchestvo Nevrologov [i] Vserossiiskoe Obshchestvo Psikhiatrov*, 119(3), pp.62.

[15] Trishchynska, M. A., Globa, M. V., Orzheshkovskyi, V. V., Voronenko, Y. V., & Mykhalchuk, V. V. (2019) "Assessment of Endothelial Function in Patients with Initial Manifestations of Chronic Cerebral Ischemia", *Wiadomoci Lekarskie (Warsaw, Poland: 1960)*, 72(10), pp.1924-1929.

[16] Pang, Q., Wu, Q. Q., Hu, X. X., Zhang, J. J., & Jiang, Q. S. (2020) "Tooth Loss, Cognitive Impairment and Chronic Cerebral Ischemia", *Journal of Dental Sciences*, 15(1), pp.84-91.

[17] Eugster, H. P., Probst, M., Würgler, F. E., & Sengstag,C. (2016) "Caffeine, Estradiol, and Progesterone Interact with Human Cyp1a1 and Cyp1a2. Evidence from Cdna-Directed Expression in Saccharomyces Cerevisiae", *Drug Metabolism & Disposition*, 21(1), pp.43-49.

[18] None. (2018) "Body Composition and Bone Mineral Density after Ovarian Hormone Suppression with or without Estradiol

Treatment: Erratum", *Menopause*, 25(3), pp.359.

[19] Gao, R., Cui, X., Hao, Y., Zhang, L., Liu, D., & Tang, Y. (2016) "A Highly-Efficient Imprinted Magnetic Nanoparticle for Selective Separation and Detection of 17â-Estradiol in Milk", *Food Chemistry*, 194(MAR.1), pp.1040-1047.

[20] Zhang, L., Xiong, W., Xiong, Y., Liu, H., Li, N., Du, Y., & Liu, Y. (2016) "Intracellular Wnt/Beta-Catenin Signaling Underlying 17beta-Estradiol-Induced Matrix Metalloproteinase 9 Expression in Human Endometriosis", *Biology of Reproduction*, 94(3), pp.70.

[21] None. (2016) "Correction: Novel Antitumor Effect of Estradiol in Athymic Mice Injected with a t47d Breast Cancer Cell Line Overexpressing Protein Kinase c Alpha", *Clinical Cancer Research*, 22(18), pp.4756-4756.

[22] Kobayashi, K., Tamotsu, S., Yasuda, K., & Oishi, T. (2016) "Vitellogenin-Immunohistochemistry in the Liver and the Testis of the Medaka, OryziasLatipes, Exposed to 17beta-Estradiol and p-Nonylphenol",*Zoological Ence*,22(4), pp.453-461.

[23] Tilton, J., E. Biggs, C., Hunter,M. G., & Foxcroft, G. R. (2018) "Gonadotropin Secretion in Ovariectomized Chinese Meishan and Hybrid Large White Gilts; Responses to Challenges with Estradiol Benzoate, Gonadotropin-Releasing Hormone, or Porcine Follicular Fluid", *Alcohol*, 66(5), pp.963.

[24] Kim, J., Szinte, J. S., Boulware, M. I., & Frick, K. M. (2016) "17â-Estradiol andAgonism of g-Protein-Coupled Estrogen Receptor Enhance Hippocampal Memory Via Different Cell-Signaling Mechanisms", *Journal of Neuroence the Official Journal of the Society for Neuroence*, 36(11), pp.3309.

[25] Oberlander, J. G., & Woolley, C. S. (2016) "17â-Estradiol Acutely Potentiates Glutamatergic Synaptic Transmission in the Hippocampus through Distinct Mechanisms in Males and Females", *Journal of Neuroence the Official Journal of the Society for Neuroence*, 36(9), pp.2677.