

***In vitro* experimental study of delayed intracranial hemorrhage due to vitamin K deficiency in mice infected with cytomegalovirus.**

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Abstract

Objective: To investigate the changes of learning and memory function and neurological function in mice with intracranial hemorrhage caused by delayed vitamin K deficiency induced by cytomegalovirus infection.

Methods: Intracranial hemorrhage mice model (model group, n=10) that was infected with cytomegalovirus due to vitamin K deficiency was conducted. At the same time, the mice that had matched age and weight with those of the model group were selected as normal control group (n=10). Mice learning and memory performance was measured by Morris water maze test when these mice were raised up to the age of 2 months and compared between the two groups. And then the score of neurological test were observed and compared in the two groups.

Results: Compared with the normal control group, the average escape latency of model group mice was significantly prolonged. The times of crossing the platform of the mice in the model group decreased significantly. At the age of 2 months, the neurological function score of the model group was 2.75 ± 0.82 , which was significantly higher than that of the control group.

Conclusion: The learning and memory ability of mice with intracranial hemorrhage caused by delayed vitamin K deficiency due to cytomegalovirus infection decreased. And there were different degrees of neurological deficits.

Keywords: Cytomegalovirus (CMV) infection, Vitamin K deficiency, Intracranial hemorrhage, Mice, Neurological function.

Accepted on October 18, 2017

Introduction

Human Cytomegalovirus (CMV) is a relatively common intrauterine infection pathogen [1]. If woman is infected with human cytomegalovirus, which can cause intrauterine infection and cause the risk of miscarriage or deformity in the fetus in her early stage of pregnancy. Related data showed that [2,3] some new-born infants infected with human cytomegalovirus may not have obvious symptoms at birth, however, they may have problems like dysuria in the long run. Some data also showed that [4,5] the late vitamin K deficiency is associated with human cytomegalovirus infection. Cytomegalovirus infection may cause certain damage to the liver, lung, brain and other organs of people. So, they should be paid great attention to and treated as soon as possible. In clinical trials, it is not uncommon for intracranial hemorrhage patients infected with CMV inducing late vitamin K deficiency. However, there are few reports about the changes of learning and memory function and neurological function in these children. By using animal experiment, this study was to observe the cytomegalovirus infection induced by vitamin K deficiency in intracranial hemorrhage and neurological function of learning and memory in mice, to provide theoretical reference for the diagnosis and

treatment of cytomegalovirus infection caused by intracranial hemorrhage.

Materials and Methods

Experimental animal

Twenty new-born mice that were Kunming species, clean class, male, healthy, body mass (8.2 ± 2.1 g) and screened by ELISA with no cytomegalovirus infection were selected as the objects. All the mice were provided by our laboratory animal research center.

Establishment of animal model

Ten mice injected with 10 μ L human blood containing cytomegalovirus were selected randomly and then they were given CMV-DNA urine test after one month. If the mice were positive infection and late vitamin K deficiency intracranial hemorrhage after being tested, they would be brought into intracranial hemorrhage mouse model (model group) that were infected by cytomegalovirus infection induced by delayed vitamin K deficiency. Another 10 mice were chosen and each of them was injected with 10uL physiological saline with the

same method as the normal control group. All mice were kept with normal feed and free tap water. When they were fed to the age of 2 months old, they would be brought for experiment.

Learning and memory achievement test

Morris water maze test was used to measure the learning and memory performance of the two groups of mice. The mouse Morris water maze tracking system (made by Shanghai Xin Ruan Mdt InfoTech Ltd.) was used to test and record the whole trip time, the number of entering quadrants and other academic data.

Neurological function score

According to the Bederson standard for evaluation of neural function [6], the neurological function of the two groups' mice was scored: (1) 0 points: no neurological deficit, normal activities; (2) 1 points: forelimb flexion, no other abnormalities; (3) 2 points: forelimb flexion and lateral pushing resistance decreased, no spontaneous circling; (4) 3 points: the same symptom with 2 points, with spontaneous circling.

Observation of hematoma morphology

After the last neurological score, the model group mice were anesthetized with 10% chloral hydrate. Then their heart cavity was perfused rapidly with 0.9% saline 20 ml at first and fixed with 4% paraformaldehyde phosphate buffer. Craniotomy, their brains were collected and were put into 4% polyphosphate phosphate buffer solution fixed in the closed overnight. After that, their brains were placed into 20% sucrose solution. When the specimens were removed after sinking frozen section, it's time to do coronary sections before isometric to 1.0 mm for the interval from the rear, the thickness of 20 μm. What should be paid attention to was that the diameter of hematoma measured by injection of slice. The sections were stained with conventional HE (Hematoxylin Eosin).

Six mice were randomly selected from the model group and the control group. Their brains were taken immediately after excessive anesthesia, and were stored in a -30°C low temperature refrigerator. After 20 mins, the coronal slices were cut into slices with the thickness of 1 mm. And the morphology of hematoma was observed under a microscope.

Statistical analysis

SPSS 19.0 software was used to deal with the data, and the t test was used to compare the data between the two groups. The difference is statistically significant when P<0.05.

Results

Pathological observation in model group

The brain tissue of model group mice was stained with HE, and their histological changes were observed during the observation period: in the right caudate nucleus, the brain

tissues appeared in hemorrhagic area, and their edema zone was seen near the bleeding area. The cells in the bleeding area were basically necrotic and could be seen scattered in the living nerve cells. The number of neurons around the hemorrhage area decreased obviously, and bleeding was obviously seen near the blood vessels.

Gross observation showed: Model group mice in the coronal section of human blood at the injection of CMV, there were obvious bleeding areas in the right caudate nucleus. And they showed round, oval or irregular in shape (Figure 1), and the average diameter of hematoma in mice was 1.26 ± 0.3 cm. The symptoms of model group mice were like those of neurological deficit in mice.

There were no obvious changes and hematoma in the brain tissues of the normal control group. In the model group, there were different degrees of destruction and edema in the cerebral hemorrhage area and its surrounding brain tissues (Figure 2).

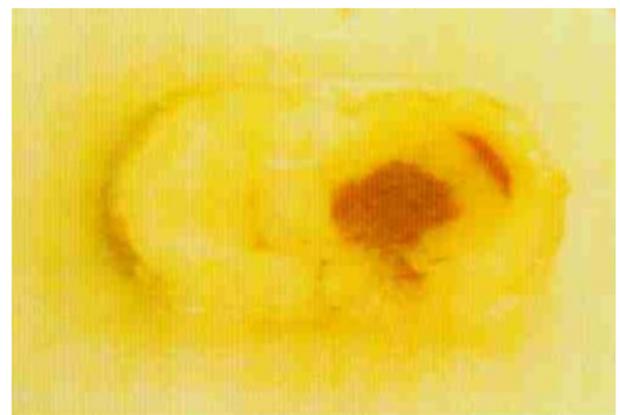


Figure 1. Hemorrhage of right caudate nucleus in mice.



Figure 2. Coronal section of brain tissue. I. Normal control group; II. Model group.

Comparison of the two groups of learning and memory performance

Compared with the normal control group, the average escape latency of the model group mice was significantly prolonged. And the model group mice's number of crossing platforms was significantly reduced (Table 1).

Table 1. Comparison of water maze results of rats in each group.

Group	n	Average latency (s)	escape	Number of traversing platforms (times)
Control group	10	21.47 ± 12.45		4.82 ± 1.34

Model group	10	43.06 ± 15.13	1.51 ± 0.65
t		8.061	7.132
P		0.029	0.015

Comparison of neurological function scores between the two groups

At the age of 2 months old, the neurological function score of the model group was 2.75 ± 0.82 , which was significantly higher than that of the control group (0.54 ± 0.03) (Figure 3).

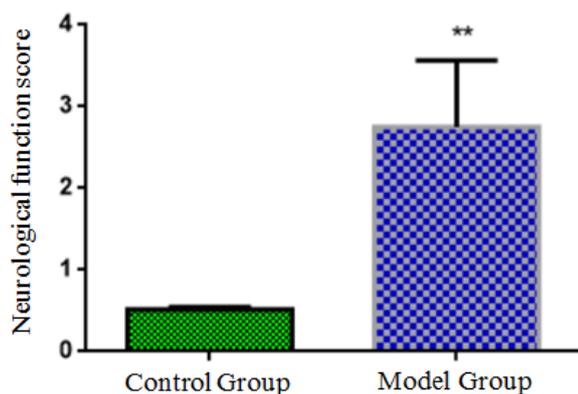


Figure 3. Comparison of neurological function scores between the two groups.

Discussion

In this study, we developed a delayed intracranial hemorrhage model of vitamin K deficiency induced by cytomegalovirus infection in mice. According to pathological observation and observation of the hematoma morphology, it was found that the hemorrhage area was appeared at the right caudate nucleus in the model group mice. What's more, the edema zone could be seen near the bleeding area. The average hematoma diameter of the mice was 1.26 ± 0.3 cm. The symptoms of model group mice were like those of neurological deficit in mice. Different degrees of destruction and edema were appeared in the cerebral hemorrhage area. Besides, surrounding brain tissue also appeared in the model group. There were no obvious changes and hematoma in the brain tissue of the normal control group, which indicated that this study successfully constructed an intracranial hemorrhage model with delayed vitamin K deficiency induced by CMV infection.

Learning and memory is a kind of high physiological activity of brain, which is the main component of cognitive activity [7,8]. With the expectations to test their cognitive function during the observation period [9,10], Morris water maze test was used to measure the spatial memory performance of animals. Therefore, the Morris water maze was used to evaluate the learning and memory ability of the mice in this study. The results showed that the average escape latency of the model group mice was significantly prolonged compared with the normal control group. And the number of platform trips was significantly reduced. The results also showed that

the intracranial hemorrhage mice caused by delayed vitamin K deficiency induced by cytomegalovirus infection was associated with low intelligence and backward movement. It is suggested that children with CMV infection should be given early intervention and prevention of mental retardation and physical retardation.

Patients with intracranial hemorrhage are prone to secondary neurological deficits for a period after the onset of the disease, while these secondary neurological deficits are visually characterized by changes in neurological function scores [11-15]. The results in this study showed that the neurological function score of the model group was 2.75 ± 0.82 at the age of 2 months old, which was significantly higher than that of the control group (0.54 ± 0.03). This shows that the blood clot produced in the bleeding area can suppress the nearby brain tissue after intracerebral hemorrhage, thus inducing partial neurological impairment in mice. It is suggested that the prevention and treatment of neurological impairment should be strengthened at children's early stage with CMV infection.

In summary, this study used the method of injecting human blood containing cytomegalovirus can be successfully constructed the mice model of intracranial hemorrhage caused by cytomegalovirus infection induced by vitamin K deficiency. This model has high feasibility and its experimental results are more objective and reliable. Cytomegalovirus infection induced by vitamin K deficiency of intracranial hemorrhage would decrease mice's ability of learning and memory and cause neurological impairment in different degrees, which has a certain reference value for the study on the pathogenesis of delayed intracranial hemorrhage due to vitamin K deficiency induced by cytomegalovirus infection.

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