0163-4984/99/71-72-0617 $11.25

Determination of Zinc Contents in Rabbits with Cerebral Ischemia by NAA and ICP-AES

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ABSTRACT

Cerebral ischemia is an important cerebral vascular disease, and zinc is a necessary trace element for humans. In this work, a cerebral ischemia model of rabbit was established by operation. The samples of brain and serum in the animal models were collected. The Zn contents in the samples were determined by neutron activation analysis and inductively coupled plasma–atomic emission spectrometry. The results show the Zn contents in brain decreased 2 mo after cerebral ischemia, and Zn contents in serum decreased even more obviously. In addition, a positive correlation of Zn contents between left and right cerebral hemispheres was observed, and the positive correlation between brain and serum was also observed. A test of Chinese medicine was also carried out in the work. Two Chinese medicines were fed to rabbits with cerebral ischemia in the experiments. The results showed they probably can prevent the decrease of Zn contents in serum.

Index Entries: Cerebral ischemia; NAA; ICP-AES; rabbit; zinc; brain, serum; Chinese medicine.

INTRODUCTION

Cerebral ischemia is a kind of important cerebral vascular disease and has high incidence. Many doctors and scientists have paid great attention

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to the disease *(1)*. The reduction of nutriional supply and disturbance of metabolism caused by cerebral ischemia can change the elemental contents in blood and brain. Zinc is a necessary trace element for humans. It is the component part of various enzymes and an important element that affects the brain function *(2,3)*. Therefore, a study on the changes of Zn contents and its distribution after cerebral ischemia may provide useful information for the etiological research and cure of the disease.

Rabbits have been used in research of cerebral ischemia as an animal model *(4)*. In the present study, we established a cerebral ischemia model of rabbit by operation. The samples of brain and serum in the animal models were collected after cerebral ischemia at different time intervals of up to 2 mo. The Zn contents in the brain were determined by neutron activation analysis (NAA), and the Zn in serum was determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES).

Traditional Chinese medicine is an effective method to cure cerebral ischemia. Two Chinese medicines, the main composition of which is the leech *(5)*, were used with the animal model, and their function was observed.

MATERIALS AND METHODS

New Zealand white rabbits weighting about 2.5 kg were used in the experiments. The cerebral ischemia models of rabbits were established by operation. In the operation, the cerebral arteries of the rabbits were blocked. The rabbits were sacrificed at different periods up to 2 mo after the operation, and samples of the brain and serum were collected.

The controls for brain samples came from normal rabbits under the same breeding conditions. In order to keep the same conditions between experiment groups and control groups, similar operations were carried out on the conrol groups. In those operations, the cerebral artery was not blocked.

The controls for serum samples came from an identical rabbit before the operation. Previous experiments indicated that at constant conditions, Zn contents in serum of normal rabbits did not change before and 1–2 mo after similar operation.

The Zn contents in brain were determined by NAA. The determinations were carried out at the Shanghai Institute of Metrology and Testing Technology. The 100-mg lyophilyzed brain samples were irradiated in a Miniature Neutron Source Reactor (MNSR) with a neutron flux of 5×10^{11} n/cm^2 s for 18 h. The 1115-keV γ-peak of ^{65}Zn was measured for 1 h after 1–3 months decay. Details of the NAA procedure have been given previously *(6)*.

The Zn contents in serum were determined by ICP-AES. Samples of 0.5 mL serum was decomposed with nitric acid and perchloric acid, and

then the Zn contents were determined with an inductively coupled argon plasma (ICAP) type 9000 plasma spectrograph.

The quality of analysis was checked by analyzing standard reference materials Bovine Liver NBS 1577a and Human Hair GBW 09101. The results showed the analytical procedures were reliable.

In the Chinese medicine test, two new Chinese medicines, medicine A and B, were used in the experiments. Medicine A is a single-drug prescription made from leech. Medicine B is a compound prescription, its main composition is also leech. The leech has the function of promoting blood circulation according to Chinese medicine. The medicines were fed to rabbits every day for 1 mo. The dose of medicines was five times as much as ordinarily given. The rabbits were sacrificed and samples were collected, and then the Zn contents measured as mentioned earlier.

In this work, every experiment group or control group included five to six cases.

RESULTS AND DISCUSSION

Table 1 lists the Zn contents in brain at different periods after inflicting cerebral ischemia. The results indicate that Zn contents in brain did not change substantially within 1 mo after cerebral ischemia, but the contents decreased substantially after 2 mo. It means that under the state of cerebral ischemia, Zn contents in brain decreased slowly, and only after a long time, the Zn contents decreased substantially. Table 2 lists the Zn contents in serum. It shows that Zn contents in serum decreased after cerebral ischemia, and the longer time, the lower the Zn contents. From the above results, Zn contents tend to decrease after cerebral ischemia both in brain and in serum, but the Zn contents in serum decreased substantially. Possibly, it is because reduction of nutritional supply after cerebral ischemia results in a decrease of Zn contents. Therefore, increasing the intake of zinc may be helpful for cerebral ischemia patients.

Tables 3 and 4 show the results of Chinese medicine test. From the two tables, we can see that after feeding the Chinese medicines to the rabbits with cerebral ischemia for 1 mo, the Zn contents did not change substantially in either the brain or serum. However, under the same conditions, for the rabbits which were not fed the medicines, the Zn contents in serum decreased substantially (see Table 2). Thus, we conjecture that the Chinese medicines can prevent the decrease of Zn contents in serum. In the test, the larger dose of medicines was used, but toxicity and side effects of the medicines were not found; thus the new medicines can probably be applied to patients.

Although the operation of establishing cerebral ischemia model was carried out in the left cerebral hemisphere, we colleced the samples in left and right cerebral hemispheres separately. The results show that the Zn contents have no obvious difference between left and right hemispheres.

Table 1
Zn Contents in Brain After Cerebral Ischemia (μg/g Dry Weight) and *t*-Test

period after cerebral ischemia	experiment group n	mean ± SD	control group n	mean ± SD	t	p
several hours	5	65.0 ± 4.1	5	63.4 ± 6.0	0.49	> 0.05
one month	5	50.4 ± 8.7	6	49.2 ± 4.5	0.30	> 0.05
two months	6	53.2 ± 3.6	5	66.7± 12.7	2.5	< 0.05

Table 2
Zn Contents in Serum After Cerebral Ischemia (μg/mL) and *t*-Test

period after cerebral ischemia	experiment group n	mean ± SD	control group n	mean ± SD	t	p
one month	5	1.4 ± 0.1	6	1.7 ± 0.2	3.0	< 0.05
two months	6	0.85 ± 0.18	6	1.5 ± 0.2	5.9	< 0.01

Table 3
Influence of Chinese Medicine on Zn Contents in Brain
(μg/g Dry Weight) and *t*-Test

chinese medicine	experiment group n	mean ± SD	control group n	mean ± SD	t	p
medicine A	6	49.8 ± 2.8	6	49.2 ± 4.5	0.28	> 0.05
medicine B	6	51.4 ± 5.2	6	49.2 ± 4.5	0.78	> 0.05

Table 4
The Influence of Chinese Medicine on Zn Contents in Serum
(μg/mL) and *t*-Test

chinese medicine	experiment group n	mean ± SD	control group n	mean ± SD	t	p
medicine A	6	2.1 ± 0.5	6	2.1 ± 0.4	0	> 0.05
medicine B	6	1.4 ± 0.3	6	1.3 ± 0.2	0.86	> 0.05

For example, for the brain samples collected 2 mo after cerebral ischemia, the Zn contents in left and right hemispheres were 53.2 ± 3.6 and 56.6 ± 6.1 μg/g, respectively. There was no obvious difference, and both of them were lower than that in controls.

The Zn contents between left and right hemispheres showed an obvious positive correlation. The correlation coefficient from 28 cases was 0.587. It means that the animals have the ability to regulate and balance, as the Zn contents in the left and right hemispheres tend to agreement. In addition, the positive correlation of Zn contents was also observed between brain and serum. For 29 cases, the correlation coefficient was 0.491.

ACKNOWLEDGMENTS

We are grateful to the teachers and doctors of Shanghai Second Medicine University for their work in breeding animals and collecting

samples. We thank Mr. Fu Youjun, Mr. Xu Kai, Mr. Xu Weixing and Mr. Sui Guoping (Shanghai Institute of Metrology and Testing Technology) for their help in NAA. We also thank Ms. He Weiyu (Shanghai Institute of Nuclear Research) for her help in the radioactivity measurements.

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