MEDIRES
Symbol of Quality Research

Research of Gastric Management and Hepatology
info@mediresonline.org

ISSN: 2836-6204 RESEARCH ARTICLE

Subtotal Stepwise Cerebral Ischemia - Methodological Approaches to Modeling and Manifestations of Neurological Deficit

Lizaveta I. Bon[1*], Maksimovich N.Ye[1]., Bon E.I[1]., Holik S.V[1]., Prykhodzka A.A[1]., Kovalenya[1]

[1]*T.A Grodno State Medical University, 80, Gorkogo St., 230009, Grodno, Republic of Belarus*

***Corresponding Author**: Lizaveta I. Bon, Candidate of biological science, assistant professor of pathophysiology department named D.A. Maslakov, Grodno State Medical University.

Received Date: 02 Februvary 2023; **Accepted Date:** 09 Februvary 2023; **Published date:** 21 Februvary 2023

Citation: Lizaveta I. Bon, Maksimovich N.Ye, Bon E.I., Holik S.V, Prykhodzka A.A, et.al (2023) Subtotal Stepwise Cerebral Ischemia - Methodological Approaches to Modeling and Manifestations of Neurological Deficit. Research of Gastric Management and Hepatology. 2(1). DOI: 10.58489/2836-6204/005

Abstract

The invention relates to experimental medicine and biology and is devoted to the development of a new method for modeling ischemic brain damage, which is a common and severe pathology that causes not only high disability, but mortality. The proposed method of modeling subtotal ischemia by stepwise (non-momentary) ligation of both common carotid arteries has an advantage over the prototype since it does not lead to the death of experimental animals, which allows to study the consequences of severe cerebral ischemia and the pathogenesis of ischemic injuries on the brain in dynamics, to develop corrective measures.

Keywords: *subtotal stepwise cerebral ischemia; methodological approaches; neurological deficit*

Introduction

The invention relates to experimental medicine and biology and is devoted to the development of a new method for modeling ischemic brain damage, which is a common and severe pathology that causes not only high disability, but mortality.

Various methods of modeling cerebral ischemia are known, including in rats: complete (total) ischemia (achieved by decapitation, cardiac arrest or occlusion of the aorta and vena cava), incomplete (subtotal) ischemia (occlusion of both common carotid arteries with intracranial hypertension), partial (partial) ischemia (occlusion of the common carotid artery), focal (local) ischemia (occlusion of the middle cerebral artery or its embolization by macrospheres), multifocal cerebral ischemia (multiple embolization by microspheres) [4,5,6,8,9,11].

Many of these methods involving occlusion of the distal branches of the willis circle are complex, traumatic, and require special expensive equipment.

Most often, partial and total ischemia models are used to study the effects of ischemia on the brain due to the possibility of performing them without special equipment. Unilateral ligation of the carotid artery leads only to a decrease in blood circulation of the brain due to the closed Willisian circle in rats, and simultaneous bilateral ligation of the common carotid arteries, carrying up to 90% of blood to the brain, leads to rapid death of animals (within 2 hours). In this regard, the model is not suitable for studying severe ischemic effects in dynamics. For a detailed study of the mechanisms of damage development and adaptation in the brain, it is necessary to develop an adequate model that would increase their life expectancy after surgery.

The purpose of the invention is to develop a less traumatic method of cerebral ischemia modeling, which allows studying the dynamics of damage and adaptive mechanisms of the brain and does not cause rapid death of animals.

The proposed method of modeling cerebral ischemia (CI) is carried out by stepwise ligation of the common carotid arteries (CCA) in rats. At the first stage, partial cerebral ischemia is modeled by dressing one CCA. At the second stage (after 3 or more days), subtotal CI is modeled by dressing the second CCA, so both CCA are bandaged, but not simultaneously, as in the prototype, but with a break of three or more days. With this method of modeling, animals are stable and do not die. It is possible to use a shorter time interval between dressings. However, its reduction to 2 or 1 days contributes to the death of some experimental animals after the second dressing of the CCA and

shortening the life of the surviving animals. The falsely operated animals of the control group (without CCA dressing) did not die, and the animals that underwent simultaneous dressing of both WASPS (simultaneous subtotal CI) died within 2 hours. So, the simulation of CI by non-simultaneous (stepwise) dressing of the CCA contributed to the survival of animals, unlike rats with simultaneous dressing (subtotal CI model).

When implementing the proposed method, there is no need to use special equipment during the period of surgical intervention, and the traumatism of surgical intervention is also reduced, which contributes to the absence of death of rats in the group of experimental animals and the absence of pronounced neurological deficit in them.

What is new in the proposed method of modeling stepwise CI is the prolongation of the life of animals after surgery, which makes it possible to assess the nature of damage and adaptive mechanisms of the brain in dynamics.

Distinctive features have shown in the claimed totality new properties that do not explicitly follow from the state of the art in this field and are not obvious to a specialist. An identical set of signs has not been found in the patent and scientific-medical literature.

The present invention can be used to simulate ischemic brain injuries associated with atherosclerotic obstruction of the carotid arteries during surgical operations using artificial circulation, which will serve as a fundamental basis for improving methods of diagnosis, treatment and prevention of the effects of ischemia, as well as to deepen understanding of the pathogenesis of this pathology.

Based on the above, the proposed invention should be considered to meet the conditions of patentability "Novelty", "Inventive step", "Industrial applicability".

The method was carried out as follows.

Method

The experiments were performed on mongrel white female rats (n=28) weighing 220-240g, obtained from the vivarium of the Grodno State Medical University. The duration of the acclimatization period for all animals was 14 days. The animals were kept on free feed intake (standard ration for laboratory rats) and had free access to water. All experiments are approved by the Ethics Committee of Grodno State Medical University. Experiments on animals were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes [7]. The choice of experimental animals was determined by the similarity of the angioarchitectonics of the rat and human brains.

To simulate stepwise cerebral ischemia (SSCI), sequential ligation of one and then the second CCA was performed with an interval of 5 days. To do this, rats under intravenous thiopental anesthesia (40-50 mg/kg) were incised along the midline of the ventral surface of the neck, the left WASP was isolated and then ligated, and then, at the second stage, the right CCA (n=8). As comparison groups, rats (n=7) with partial CI were used, who were ligated only with the left CCA under similar experimental conditions, and rats with simultaneous ligation of both common carotid arteries (n=6). To assess the severity of ischemic brain damage, a number of behavioral techniques were used – the "Muscle Strength" test and the "swimming test" [2,3], as well as emotional state and motor activity were studied in the "open field" test [1,10]

Muscle strength was assessed by placing a rat on a horizontally arranged metal mesh 60 cm long with a centimeter scale of divisions applied and determining the time of holding the animal after turning the mesh (90°) vertically. To conduct a "swimming test", the animals were placed in a glass tank with water (21 ° C) and the time of holding the animal on the water surface was determined. The "open field" test was conducted according to a generally accepted methodology by evaluating such indicators as the number of crossed squares (motor activity), washing (grooming), racks (looking back reaction) and the number of acts of defecation and urination (emotionality) [1,10].

Studies were carried out in rats with CI after ligation of the left carotid artery and in rats with stepped subtotal CI after the second CCA ligation. The rats of the control group underwent a similar surgical intervention, but without applying ligatures to the vessels (n=7). The samples were performed 5 days after the false surgical intervention.

Statistical data processing was carried out using the program "Statistica 10". After checking the data for the normality of the distribution according to the Mann-Whitney criterion, nonparametric statistical methods were used, median, interquartile interval (25th and 75th percentiles) were calculated. The differences were considered statistically significant at $p < 0.05$

Result

As a result of the conducted studies, it was found that animals with partial ischemia of GM had higher muscle strength (retention time on the grid was 5

(4.7;5.9) minutes) than animals with stepped subtotal CI. Their retention time on the grid was 1 (0.45;1.2) minute, ($p<0.05$). The retention time on the grid of animals of the control group was 20 (15; 24) minutes.

They were also more resistant to hypoxia of the load in the "swimming test". During the swimming test, the retention time of rats with partial CI on the water was 12 (10.5; 12.3) minutes, while in animals with stepped subtotal CI it was 5.3 (4.5; 5.7) minutes ($p<0.05$). The swimming time of the control group animals was 21 (18;23) minutes.

During the "open field" test, the following was found: in rats with partial CI, the number of crossed squares was 54 (52;59), washings 4 (3.5;4.3), racks 6 (5.8;6.4), the number of acts of defecation and urination 3 (2.5;3.6). At the same time, in animals with stepped subtotal CI, the number of intersected squares was 24 (22;28), washings 2 (1,6;2,4), racks 3 (2,6;3,2), the number of acts of defecation and urination 2 (1,2;2,3). In animals of the control group, the number of crossed squares was 67 (64;72), washings 6 (5.1;7.2), racks 9 (8;9.4), the number of acts of defecation and urination 5 (4.6;6.5), ($p<0.05$).

In animals, simultaneous ligation of both common carotid arteries with subtotal IGM was not possible to conduct tests, since the animals were depressed and did not show activity until their death in the next few hours.

Conclusion

Thus, the proposed method of modeling subtotal CI by stepwise (non-momentary) ligation of both common carotid arteries has an advantage over the prototype since it does not lead to the death of experimental animals, which allows to study the consequences of severe cerebral ischemia and the pathogenesis of ischemic injuries on the brain in dynamics, to develop corrective measures.

References

1. Buresh, Ya. (1991) Methods and basic experiments to study the brain and behavior / Ya. Buresh, O. Bureshova, J.P. Houston // M. Higher School. -. - 400 p.
2. Maksimovich, N.E. (2017) A method for studying the muscle strength of rats by using a vertical plane / N.E. Maksimovich / / GrSMU, certificate No. –10.10.
3. Maksimovich, N.E. (2017) A method for assessing the severity of ischemic brain damage in rats by performing a swimming test. /N.E. Maksimovich// GrSMU, certificate No.–10.10.
4. Rozvadovsky, V. D. (1985) Microsurgical model of cerebral ischemia / V. D. Rozvadovsky, S. O. Trenin, V. I. Telpukhov // Journal of Pathological Physiology and General Pathology. -. - No. 2. - P. 87-92.
5. Shcherbak, N. S., Galagudza M. M., Kuzmenkov A. N. (2005) A new method for modeling reversible global cerebral ischemia // Byul. experiment biol. and honey. - 2011. - T.152, No. 11. – S. 592–59Carmichael, S. T. Rodent models of focal stroke: size, mechanism, and purpose / S. T. Carmichael // – Neuro. – V. 2. –P. 396–409
6. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes // ETS №123. – Strasbourg. 1986
7. Beech, J. S., Williams, S. C., Campbell, C. A., Bath, P. M., Parsons, A. A., Hunter, A. J., & Menon, D. K. (2001). Further characterisation of a thromboembolic model of stroke in the rat. *Brain research*, *895*(1-2), 18-24.
8. Yamaguchi, M., Calvert, J. W., Kusaka, G., & Zhang, J. H. (2005). One-stage anterior approach for four-vessel occlusion in rat. *Stroke*, *36*(10), 2212-2214.
9. Kutcher, E., Egorov, A., Filatova, E., Kulagina, K., & Chernikova, N. (2011). Sex influence on the formation of alcohol preference and behavior in rats during the long-term caffeine and ethanol intake. *Journal of behavioral and brain science*, *1*(03), 172.
10. Smrcka, M., Ogilvy, C. S., & Koroshetz, W. (2002). Small aneurysms as a cause of thromboembolic stroke. *Bratislavske lekarske listy*, *103*(7/8), 250-253.