AUCTORES
Globalize your Research

Biotechnology and Bioprocessing
Lizaveta I. Bon *

Open Access

Research Article

Neurological Deficit and Corrective Effect of Omega-3 Polyunsaturated Fatty Acids in Cerebral Ischemia in Rats: A case-control study

Lizaveta I. Bon* and Maksimovich N.Ye.

Grodno State Medical University, 80, Gorky St., 230009, Grodno, Belarus.

***Corresponding Author:** Lizaveta I. Bon, Candidate of biological science, assistant professor of pathophysiology department named D.A. Maslakov, Grodno State Medical University, Grodno State Medical University, 80, Gorky St., 230009, Grodno, Belarus.

Received date: July 30, 2021; **Accepted date:** September 16, 2021; **Published date:** October 06, 2021

Citation: Lizaveta I. Bon and Maksimovich N.Ye (2021) Neurological Deficit and Corrective Effect of Omega-3 Polyunsaturated Fatty Acids in Cerebral Ischemia in Rats: A case-control study. *J. Biotechnology and Bioprocessing*. 2(8); DOI: 10.31579/2766-2314/050

Abstract

Objectives: It was to assess vasoprotective effects of ω-3 polyunsaturated fatty acids in cerebral ischemia.

Materials and methods: The experiments were carried out on 42 male outbred white rats weighing 260 ± 20 g. modeling of cerebral ischemia was carried out under conditions of intravenous thiopental anesthesia (40-50 mg / kg). The studies used models of subtotal, partial and stepwise subtotal cerebral ischemia. The table shows the experimental groups and the number of animals in them. Subtotal cerebral ischemia (SCI) was modeled by simultaneous ligation of both common carotid arteries (CCA). Partial cerebral ischemia (PCI) was modeled by ligating one CCA on the right. Stepwise subtotal CI (SSCI) was performed by sequential ligation of both CCA with an interval of 1 day (subgroup 1), 3 days (subgroup 2), or 7 days (subgroup 3). To study the effects of omega-3 polyunsaturated fatty acids (ω-3 PUFA), animals with CI were injected intragastrically with the drug "Omegamed" (SCI+ω-3 PUFA) at a dose of 5 g / kg body weight for a week. The control group consisted of sham-operated rats of the same sex and weight. Neurological deficits were assessed in the "muscle strength", "swimming test" and "open field" tests after 5-6 hours of the ischemic period.

The study was carried out 6 hours after the simulation of the CI. Quantitative continuous data were obtained, which were processed using the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). Since the experiment used small samples that had an abnormal distribution, the analysis was carried out by methods of nonparametric statistics. Data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the upper quartile value. Differences between groups were considered significant at $p < 0.05$ (Regression Model).

Results: With a stepwise bilateral ligation of both common carotid arteries with an interval of 1 day, neurological disorders were most pronounced, which indicates an aggravation of neurological deficit with a reduction in the time between CCA dressings. In rats with SCI, the changes were more pronounced than with PCI, but less than with SCI. The least pronounced changes were noted in the 3rd subgroup (the interval between CCA dressings was 7 days). Studies have shown the dependence of the severity of brain damage in SSCI on the interval between the cessation of blood flow in both CCA. At a 7-day interval between CCA dressings, compensatory mechanisms were activated, which prevented the development of morphological changes and neurological deficits. When CCA was ligated with an interval of 1 day, the degree of neurological deficit was maximal, which indicates insufficient implementation of compensatory mechanisms. Compared with the control group, the rats of the "SCI+ω3-PUFA" group retained neurological deficit, the muscle strength indicator was 86% less ($p<0.05$), the swimming duration - by 63% ($p<0.05$), the number of crossed squares - by 55% ($p<0.05$), the number of washes - by 62% ($p<0.05$), the number of racks - by 62.5% ($p<0.05$) and the number of bowel movements - by 60% ($p<0.05$). However, in comparison with the SCI group, the neurological deficit was less pronounced. There was an increase in muscle strength by 67% ($p<0.05$), swimming duration by 37.5% ($p<0.05$) and the number of squares crossed in the open field test by 31% ($p<0.05$), which indicates the presence of a corrective action in the ω-3 polyunsaturated fatty acids preparation.

Conclusion: The introduction of the preparation of ω-3 polyunsaturated fatty acids has a corrective effect in conditions of subtotal cerebral ischemia, contributing to a lesser severity of manifestations of neurological deficit (an increase in muscle strength, duration of swimming and the number of squares crossed in the open field test).

Key words: cerebral ischemia, rats, neurological deficiency, omega-3 polyunsaturated fatty acids

Introduction

Acute disorders of cerebral circulation are one of the most pressing problems in modern medicine. The incidence of strokes varies in different regions of the world from 1 to 4 cases per 1000 population per year, increasing significantly with age. Cerebrovascular diseases of ischemic genesis tend to grow, rejuvenate, are associated with a severe clinical course, high rates of disability and mortality [1-4]. The primary mechanism by which stroke causes injury is the focal deprivation of blood flow to the cerebral parenchyma. While a variety of phenomena can result in such ischemia, large-arterial atherosclerosis is the most prevalent. In atherosclerosis accumulations of fatty material in the arterial subintima amass platelet clumps. These clumps then attract thrombin, fibrin, and erythrocyte debris that can ultimately coagulate to a size that poses stenotic risk to the cerebral vasculature. Local blood flow stagnation due to low wall shear stress is thought to predispose certain areas of the vasculature, such as the carotid bulb, to atherosclerotic plaque development. In any case, the resulting thrombus deprives cells of the cerebral parenchyma of the oxygen they need to function, causing pathology. Plaque development and succeeding stenosis are not necessarily *in situ*, however. Plaques can also travel to the cerebral circulation from another location, in which case they are called emboli. The heart, by way of atrial fibrillation, is the most common source of these, but they can come from other diseased parts of the arterial system, as well [14, 15].

There are many other pathogenic routes to cerebral ischemia. In addition to the large-vessel infarcts just discussed, which involve the carotid, vertebral, and basilar arteries, as well as major branches of the circle of Willis, small-vessel (or lacunar) infarcts are also a major etiology. Commonly by lipohyalinosis or micro-atheroma, but also occasionally by the same mechanism by which larger arteries are blocked, the blockage of these small, penetrating arteries running at right angles to the major branches produces the focal deficits characteristic of stroke. Some less frequently observed causes include acute arterial dissection secondary to fibromuscular dysplasia, hematologic disorders such as sickle cell anemia, and recreational use of cocaine or amphetamines [14,15,16,17].To study the degree of neurological and behavioral disorders in adult animals with cerebral ischemia, a number of methods can be used: Bederson's test, the test for assessing the modified depth indicators of neurological deficit, the Garcia test, the angular test, the leg extension test, the "open" test. They allow you to monitor impaired motor function, for example, to register discoordination, trembling, paresis, paralysis [5-11]. Bederson's test is as follows: the rat is held by the tail at a distance of 1 meter above the floor and the mobility of the forelimbs is monitored. Normally, rats pull their limbs towards the floor. The test involves placing the rats on a slippery smooth surface and pressing gently from the side behind the shoulder until the forelimbs begin to slide. Animals must equally resist sliding in both directions. Scale for assessing indicators of the depth of neurological deficit. This scale includes tests for detecting motor activity when hanging an animal by the tail, features of walking on a horizontal plane, coordination of movements when walking on a beam, the severity of reflexes. The Garcia test includes an assessment of spontaneous activity in the cage for 5 min, the symmetry of the stretching of the forelimbs when the animals are suspended by the tail, the ability to climb the wall of the ethmoid cage, the response to touching each side of the rat's body, response to touching vibrissae [6].

Angle test. This test evaluates space perception disorders and gaze paresis. The rat is placed between two vertical planes. Intact rats easily turn both to the right and to the left. In a number of pathologies, including cerebral ischemia, neglect is observed - while the animal is not able to perceive a certain part of the space. The "paw extension" test allows to identify and evaluate disorders of the forelimb motor activity. The limbs of the rat during the study should hang without support, then it is raised to the edge of the platform so that its vibrissae touch the surface of the plane. The animal is held by hands and pulled to the side on a smooth surface. The number of movements of the forelimbs performed on the side from which the rat is pushed is recorded. At the same time, intact rats perform many movements with their front paws [6].

In the "open field", the number of crossed squares, activity in the horizontal and vertical planes, grooming (washing), the number of bowel movements, and the search for depressions and holes for animals are assessed [7,8,9,10,11,12,13].

One of the most promising principles of modern science is the search for new methods of prevention and treatment of strokes. Omega-3 polyunsaturated fatty acids (ω-3 PUFA), such as eicosapentaenoic acid and docosahexaenoic acid, are widely regarded as vasoprotective. Several large-scale, randomized clinical trials have shown that dietary intake of omega-3 PUFAs improves the prognosis of patients with symptomatic heart failure or recent myocardial infarction. Omega-3 PUFAs can be incorporated into the phospholipid bilayer of cell membranes and can affect membrane fluidity, lipid microdomain formation, and signaling across membranes. Omega-3 PUFAs also modulate the function of membrane ion channels, such as Na and L-type Ca channels, to prevent lethal arrhythmias. Moreover, omega-3 PUFAs also prevent the conversion of arachidonic acid into pro-inflammatory eicosanoids by serving as an alternative substrate for cyclooxygenase or lipoxygenase, resulting in the production of less potent products. In addition, a number of enzymatically oxygenated metabolites derived from omega-3 PUFAs were recently identified as anti-inflammatory mediators. These omega-3 metabolites may contribute to the beneficial effects against vascular that are attributed to omega-3 PUFAs [12,13,14,16, 17,18,19].

The aim of this work was to assess vasoprotective effects of ω-3 polyunsaturated fatty acids in cerebral ischemia.

Materials and methods

The experiments were carried out on 42 3-month-old male outbred white rats weighing 260 ± 20 g. The animals were kept in an air-conditioned room (22° C) under mixed illumination on a standard vivarium ration and free access to food and water for no more than 5 animals per cage. Protocols were reviewed and approved by the Ethical Committee of the Grodno State Medical University (protocol No 1, 14.04.2013) [3]. Modeling of cerebral ischemia (CI) was carried out under conditions of intravenous thiopental anesthesia (40-50 mg / kg). The studies used models of subtotal (SCI), partial (PCI) and stepwise subtotal (SSCI) cerebral ischemia. The table shows the experimental groups and the number of animals in them (table 1).

Experimental groups		number of animals
SCI		6
PCI		6
SSCI	subgroup 1 (1 day)	6
	subgroup 2 (3 days)	6
	subgroup 3 (7 days)	6
SCI + Omega-3 PUFAs		6
control		6

Table 1 - *Experimental groups*

Subtotal cerebral ischemia (SCI) was modeled by simultaneous ligation of both common carotid arteries (CCA). Partial cerebral ischemia (PCI) was modeled by ligating one CCA on the right. Stepwise subtotal CI (SSCI) was performed by sequential ligation of both CCA with an interval of 1 day (subgroup 1), 3 days (subgroup 2), or 7 days (subgroup 3). To study the effects of omega-3 polyunsaturated fatty acids (ω-3 PUFA), animals with CI were injected intragastrically with the drug "Omegamed" (SCI + ω-3 PUFA) at a dose of 5 g / kg body weight for a week. The control group consisted of sham-operated rats of the same sex and weight. Neurological deficits were assessed in the "muscle strength", "swimming test" and "open field" tests after 5-6 hours of the ischemic period. Muscle strength and swimming test tests are used to study physical activity. The "muscle strength" test is assessed by placing the rat on a horizontal 60 cm long metal mesh with a centimeter scale of divisions and determining the retention time of the animal after turning the mesh to a vertical position [6] (Figure 1).

Figure 1 - *Assessment of the reflex "muscle strength"*

To carry out a "swimming test", the animal is placed in a glass reservoir with water (21° C) and the time of keeping the animal on the water surface is determined. The "open field" test is carried out for 5 minutes on a flat surface, lined with 36 squares, enclosed around the perimeter [6] (Figure 2).

In the "open field", the number of crossed squares, activity in the horizontal and vertical planes, grooming (washing), the number of bowel movements, and the search for depressions and holes for animals are assessed. In the "open field" it is possible to observe the violation of motor activity by registering discoordination, the disappearance of voluntary movements or their limitation. The motor activity of animals in the horizontal plane includes movement in different directions, walking in a circle. In this case, the participation in the movement of all limbs of the rat is evaluated. One crossed square is taken as a unit of movement for visual registration of activity. Motor activity of rats in the vertical plane is represented by two types of racks: slimbing (climbing) - the hind legs of the animal remain on the floor of the surface, and the front legs rest against the wall of the "open field", and rearing ("rear" - "stand on their hind legs") - the front limbs remain on weight. Grooming can be short - in the form of quick circular movements of the front paws around the nose and vibrissae, and long - washing the eyes, the area behind the ears, the entire head, paws, sides, back, anogenital region, tail. The study of holes in the floor manifests itself in sniffing at their edges or sticking a muzzle in them.[6,7,8]

Figure 2 - *Test "open field"*

The study was carried out 6 hours after the simulation of the CI. Quantitative continuous data were obtained, which were processed using the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). Since the experiment used small samples that had an abnormal distribution, the analysis was carried out by methods of nonparametric statistics. Data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the upper quartile value. Differences between groups were considered significant at $p < 0.05$ (Regression Model).

Results

The results are presented in tables.

Experimental groups		muscle strength, min	swimming test, min
Control		21(20; 23)	21,5(18;25)
SCI		1(1;1) *	5(4;5) *
SSCI	1 sg	1 (1;1) *	5 (4;5) *
	2 sg	3 (3;3) *+	8 (7;9) +*
	3 sg	4 (4;5) *+	12 (12;14) +*
PCI		5(4;5)* +	13(12;15)* +
SCI+ ω-3PUFA		3(2;3) *+	8(7;8) *+

Test "open field"

Experimental groups		number of squares crossed	number of short washes	climbing	number of acts of defecation
Control		72(64;75)	6,5(5;8)	6,5(5;8)	5(4;6)
SCI		23 (21;23)*	2(1;2) *	3(3;3)*	2(2;2) *
SSCI	1 sg	23 (21;24)*	2 (1;2) *	3 (3;3)*	2 (1;2) *
	2 sg	33 (29;33)* +	3 (2;3) *	4 (3;4)*	3 (2;3) *
	3 sg	43 (41;45) * +	3 (3;4) * +	4 (4;4)* +	3 (2;3) *
PCI		53(52;55)* +	4(3;4)* +	6(5;6)* +	3(3;3)*
SCI+ ω-3PUFA		33 (30;33)* +	3 (2;3) *	4(3;4)*	2(1;3) *

Notes (edit)

* - $p < 0.05$ compared with the control group

+ - $p < 0.05$ compared with SIGM

SCI - subtotal cerebral ischemia

SSCI - subtotal stepwise cerebral ischemia

PCI - partial cerebral ischemia

ω-3PUFA - ω-3 polyunsaturated fatty acids

sg - subgroup

Table 2 - *Indicators of changes in motor function in rats with cerebral ischemia, Me (LQ; UQ)*

percentage with the group control				
Experimental groups		muscle strength	swimming duration	number of crossed squares
SCI		95% ↓	76% ↓	64% ↓
SSCI	1 sg	95% ↓	77% ↓	68% ↓
	2 sg	86% ↓	63% ↓	55% ↓
	3 sg	81% ↓	45% ↓	40% ↓
PCI		75% ↓	41% ↓	26% ↓
SCI+ ω-3PUFA		86% ↓	63% ↓	55% ↓

Notes (edit)

* - $p < 0.05$ compared with the control group

+ - $p < 0.05$ compared with SIGM

SCI - subtotal cerebral ischemia

SSCI - subtotal stepwise cerebral ischemia

PCI - partial cerebral ischemia

ω-3PUFA - ω-3 polyunsaturated fatty acids

sg - subgroup

Table 3 - *Indicators of percentage changes with the group control*

Discussion

Thus, there were no differences in the degree of neurological deficit between single-stage SCI and the 1st subgroup of SSCI with a 1-day interval between dressings ($p>0.05$). With a stepwise bilateral ligation of both common carotid arteries with an interval of 1 day, neurological disorders were most pronounced, which indicates an aggravation of neurological deficit with a reduction in the time between CCA dressings. In rats with SCI, the changes were more pronounced than with PCI, but less than with SCI. The least pronounced changes were noted in the 3rd subgroup (the interval between CCA dressings was 7 days). Studies have shown the dependence of the severity of brain damage in SSCI on the interval between the cessation of blood flow in both CCA. At a 7-day interval between CCA dressings, compensatory mechanisms were activated, which prevented the development of morphological changes and neurological deficits. When CCA was ligated with an interval of 1 day, the degree of neurological deficit was maximal, which indicates insufficient implementation of compensatory mechanisms. So, the rats with the experimental CI had less muscle strength, showed less physical activity, and they showed behavioral disorders. The morphological basis of the revealed changes in CI is damage to the neurons of the brain as a result of the destabilization of nervous processes (the ratio of the reactions of excitation and inhibition), which affects the implementation of brain functions. In animals with SCI and in the 1st subgroup "SSCI" more pronounced disorders were observed in comparison with the 3rd subgroup "SSCI" and the group "PCI". It is obvious that with these methods of modeling CI, adaptation processes occur that prevent the development of pronounced morphological changes and allow neurons to adapt to conditions of moderate hypoxia. According to the literature, 7 days after hypoxia caused by CCA ligation, due to the development of compensatory mechanisms, there is a tendency to improve microcirculation: capillary patency is restored, their number and diameter increase, which leads to an improvement in cerebral blood flow, which is one of the important compensation effects. It is based on an increase in the density of blood vessels [3]. The corrective effect of polyunsaturated fatty acids on the state of neurons under conditions of subtotal cerebral ischemia may be due to an improvement in the rheological properties of blood due to a decrease in the production of thromboxane A by platelets and an increase in the level of tissue plasminogen activator, as well as an improvement in the fluidity of the neuronal membrane, and a decrease in blood viscosity. Not all Omega-3 PUFAs trials have shown reductions in vascular disorders. However, several adequately powered observation and intervention trials have strongly supported the efficacy of Omega-3 PUFAs for the prevention of vascular disorders. Furthermore, experimental studies have revealed multiple underlying molecular mechanisms, including membrane modification, attenuation of ion channels, regulation of pro-inflammatory gene expression, and production of lipid mediators. It remains unclear which mechanism contributes the most to the cardioprotective effects of Omega-3 PUFAs observed in vivo; however, the pleiotropic anti-inflammatory effects of Omega-3 PUFAs could be valuable, especially in the setting of atherosclerosis and cardiac remodeling. Although further work is needed to clarify the molecular relationship between Omega-3 PUFAs and vascular pathology, it might be useful to consider bioactive Omega-3 PUFA-derived metabolites, such as 18-hydroxyeicosapentaenoic acid, as endogenous anti-inflammatory molecules and potential new therapeutic targets for vascular disorders [17, 18, 19].

Omega 3-PUFAs also have an anti-inflammatory effect due to their incorporation into the phospholipid layer of cell membranes of monocytes, leukocytes, endothelial cells, which is accompanied by a decrease in the production of inflammatory mediators and a decrease in the adhesion of leukocytes to the endothelial wall. In addition, polyunsaturated fatty acids, influencing the synthesis of prostaglandins, regulate vascular tone and prevent vasoconstriction of blood vessels under the influence of catecholamines, which causes a moderate hypotensive effect [15, 16].

Conclusion

Thus the severity of neurological deficit depends on the severity of the ischemic injury. The most severe consequences occurred with subtotal one-stage ischemia and stepwise ischemia with a minimum 1 day interval between arterial ligation. Stepwise ischemia with an interval between dressings of 7 days and partial ischemia did not lead to such pronounced disorders of the neurological status. The introduction of the preparation of ω-3 polyunsaturated fatty acids has a corrective effect in conditions of subtotal cerebral ischemia, contributing to a lesser severity of manifestations of neurological deficit.

Acknowledgments

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources

State scientific program «To study the processes of damage and adaptation of the brain during its ischemia and the use of correction».

References

1. Sveinsson OA, Kjartansson O, Valdimarsson E. (2014) Cerebral ischemia/infarction - epidemiology, causes and symptoms. *Laeknabladid*. 100(5):271-279.
2. Schaar K. (2010) Functional assessments in the rodent stroke model. *Experimental & Translational Stroke Medicine*. **2**: 13–18.
3. Bon LI, Maksimovich NYe, Zimatkin SM. (2018) Effects of experemental cerebral ishemia on metabolic characteristics of parietal cortex neurons. *Bioprocess Engineering*. 1:1-5.
4. Bon LI, Maksimovich NYe, Zimatkin SM. (2020) Morphological disorders of neurons in the hippocampus of rats with subtotal and total ischemia. *Orenburg Medical Bulletin*. 2:41-46.
5. Bod'ová K. (2018) Probabilistic models of individual and collective animal behavior. *PLoS One*. 7:13-16.
6. Bon LI, Maksimovich NYe. (2019) Methods of estimation of neurological disturbances in experimental cerebral ischemia. *Biomedicine*. 2: 69-74.
7. Chouinard-Thuly L. (2017) Technical and conceptual considerations for using animated stimuli in studies of animal behavior. *Curr Zool*. 63:5-19.
8. Cinque S. (2018) Behavioral Phenotyping of Dopamine Transporter Knockout Rats: Compulsive Traits, Motor Stereotypies, and Anhedonia. *Front Psychiatry*. 22:9-43.
9. Fashing PJ. (2011) Behavior toward the dying, diseased, or disabled among animals and its relevance to paleopathology. *Int J Paleopathol*. 1:128-129.
10. Rosińczuk J. (2018) The protective action of tocopherol and acetylsalicylic acid on the behavior of rats treated with dioxins. *Adv Clin Exp Med*. 27:5-14
11. Sestakova N. (2013) Determination of motor activity and anxiety-related behaviour in rodents: methodological aspects and role of nitric oxide. *Interdisciplinary Toxicology*. 6:126–135.
12. Kaliannan K, Li XY., Wang B, Pan Q, Chen CY., Hao L, Xie S, Kang JX. (2019) Multi-omic analysis in transgenic mice implicates omega-6/omega-3 fatty acid imbalance as a risk factor for chronic disease. *Commun Biology*. 1:276-280.
13. Khunt D, Shrivas M, Polaka S, Gondaliya P, Misra M. (2020) Role of Omega-3 Fatty Acids and Butter Oil in Targeting Delivery of Donepezil Hydrochloride Microemulsion to Brain

via the Intranasal Route: a Comparative Study. *Pharmacology Sciencific Technology*. 21:45-50.

14. Mozaffarian D, Wu JH. (2011) Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol*. 58: 2047-2067
15. Tavazzi L, Maggioni AP, Marchioli R, Barlera S. (2008) Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet*. 372:1223-1230
16. Yokoyama M, Origasa H. (2007) Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet*. 369: pp. 1090-1098
17. Kris-Etherton P, Harris W, Appel L. (2002) American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 106: 2747-2757.

DOI: 10.31579/2766-2314/050