Vasoprotective Effects of Omega-3 Polyunsaturated Fatty Acids in Cerebral Ischemia

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Abstract

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

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 5g/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.*

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).*

Keywords: 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]. 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]. One of the promising ways to increase the effectiveness of the therapy of cerebral ischemia is to activate compensatory mechanisms, which determines the feasibility of conducting research in this direction. Omega-3 PUFAs ensure the functioning of cell membranes, transmembrane ion channels, are involved in the regulation of physiological processes and the implementation of the main functions of neurons - the transmission of impulses and the work of receptors. It is known that brain neurons are electrically active cells rich in ion channels, and therefore they can be sensitive to their deficiency [12, 13].

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

Methods

The experiments were carried out on 42 male outbred white rats weighing 260 ± 20 g in compliance with the Directive of the European Parliament and of the Council No. 2010/63/EU of 22.09.2010 on the protection of animals used for scientific purposes. The choice of experimental animals is due to the similarity of the angioarchitectonics of the rat and human brain. 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 5g/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 (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 (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.

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 (Kruskell-Wallis test with Bonferoni's correction).

Results

When assessing the neurological deficit in animals with SCI, there was a decrease in "muscle strength" by 95% (p<0.05), the duration of retention in the water in the "swimming test" decreased by 76% (p<0.05), Table 2.

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)*

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

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

Assessment of motor activity in the open field test also confirmed the development of neurological deficits. When conducting this study, in comparison with the indicators in animals of the control group, the number of crossed squares decreased by 64% (p <0.05), the number of short washes - by 67% (p<0.05), the number of racks - by 62.5% (p<0.05), the number of acts of defecation - by 60% (p<0.05). Compared to the rats of the control group, the animals with PCI showed a decrease in the "muscle strength" indicator and the duration of swimming by 75% (p<0.05) and 41% (p<0.05), respectively. In the open field test, the number of crossed squares decreased by 26% (p<0.05), the number of short washes - by 33% (p<0.05), the number of "climbing" racks - by 25% (p<0.05), the number of acts of defecation - by 40% (p<0.05). Prolonged washing and rearing was observed only in intact animals (p>0.05). The results of behavioral tests indicate the development of a minor neurological deficit in rats with PCI. Compared with the "control" group, in the 3rd subgroup of SSCI (interval of 7 days), the "muscle strength" indicator decreased by 81% ($p<0.05$), the duration of swimming - by 45% ($p<0.05$), the number of crossed squares in the open field test - by 40% ($p<0.05$), the number of washes - by 54% ($p<0.05$), the number of climbing racks - by 50% ($p<0.05$), the number of acts of defecation - by 40% ($p<0.05$). In the 2nd and 1st subgroups, the changes were more pronounced. So, the indicator of "muscle strength" decreased by 86 ($p<0.05$) and 95% ($p<0.05$), swimming duration - by 63% ($p<0.05$) and 77% (p<0, 05), the number of squares crossed - by 55% ($p<0.05$) and 68% ($p<0.05$), the number of washes - by 54% ($p<0.05$) and 69% ($p<0.05$), the number of climbing racks - by 57% ($p<0.05$) and by 62.5% ($p<0.05$), the number of acts of defecation - by 50% ($p<0.05$) and by 60% ($p<0.05$), respectively. Compared with the 3rd subgroup of SSCI, in the second subgroup the indicator of "muscle strength" decreased by 24% ($p<0.05$), the duration of swimming - by 33% ($p<0.05$), the number of squares crossed in the test "open field"- by 24% ($p<0.05$), and in the 1st subgroup these indicators decreased to the greatest extent - by 75% ($p<0.05$), by 58% ($p<0.05$), by 47% ($p<0.05$), respectively. In addition, in the 1st subgroup, there was a decrease in the number of washes - by 33% ($p<0.05$), the number of climbing racks - by 25% ($p<0.05$) and the number of acts of defecation - by 33% ($p<0.05$), and compared with the 2nd subgroup, there was a decrease in muscle strength by 67% ($p<0.05$), swimming duration - by 37.5% ($p<0.05$) and the number of crossed squares in open field test - by 29% ($p<0.05$). In the 3rd subgroup of SCI, the indicator of muscle strength and duration of swimming, compared with the "PCI" group, did not change ($p>0.05$), but during the "open field" test, a decrease in the number of crossed squares was observed - by 19% ($p<0.05$) and the number of racks - by 33% ($p<0.05$). Compared with SCI, in the third subgroup of SCI with an interval between dressings of both CCA for 7 days, the muscle strength indicator was 75% higher ($p<0.05$), the duration of swimming - by 58% ($p<0.05$), the number of crossed squares - by 48% ($p<0.05$), the number of washes and climbing racks - by 33% ($p<0.05$). In the first and second subgroups of SCI, the manifestations of neurological deficit were more pronounced than in rats with PCI: muscle strength index - by 40% ($p<0.05$) and 80% ($p<0.05$), swimming duration - by 39% ($p<0.05$) and 62% ($p<0.05$), the number of squares crossed - by 39% ($p<0.05$) and 57% ($p<0.05$), the number of racks climbing - by 42% ($p<0.05$) and 50% ($p<0.05$), respectively. The number of washings and defecations in the 2nd subgroup did not differ from the values of indicators in the PCI group ($p>0.05$), but in the 1st subgroup their number was 50% less ($p<0.05$). In the 2nd subgroup of SSCI, in comparison with the group SCI, the indicator of muscle strength was 67% more ($p<0.05$), the duration of swimming - by 37.5% ($p<0.05$), the number of squares crossed - by 31% ($p<0.05$) and washing - by 33% ($p<0.05$).

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. 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 ω3-PUFA preparation. 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. 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 [12, 13].

Conclusion

Thus, 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).

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Conflict of interest statement

The authors declare no conflict of interest.

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