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Morphological changes in the neurons of the parietal cortex and hippocampus of rats with subtotal cerebral ischemia under conditions of the use of modulators of the L-arginine-NO pathway and against the background of the administration of Omega-3 polyunsaturated fatty acids

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Abstract:

Cerebrovascular diseases of ischemic genesis tend to increase, rejuvenate, are associated with severe clinical course, high rates of disability and mortality. The urgency of the problem of cerebrovascular diseases can rightfully be defined as extraordinary, requiring the concentration of efforts of specialists of different profiles to solve it. Subtotal cerebral ischemia leads to the development of morpho functional disturbances of the cerebral cortex. The introduction of Omega-3 polyunsaturated fatty acids has a corrective effect on the hippocampus in conditions of subtotal ischemia, reducing the number of shadow cells and hyperchromic shrunken neurons, without significantly affecting the size and shape of neurons in the cerebral cortex. Prior administration of L-NAME, the use of Omega-3 did not prevent the effects of the NO synthase inhibitor and associated NO deficiency at this dose and route of administration.

Key words: neurons; cerebral ischemia; L-arginine; Omega-3 PUFAs

Introduction

Cerebrovascular diseases of ischemic genesis tend to increase, rejuvenate, are associated with severe clinical course, high rates of disability and mortality [1-8]. The urgency of the problem of cerebrovascular diseases can rightfully be defined as extraordinary, requiring the concentration of efforts of specialists of different profiles to solve it [2-8].

The search for new approaches to the treatment of acute ischemic stroke is one of the urgent problems of experimental and clinical neurology [1-8].

L-arginine is one of the promising neuroprotective amino acids [3,4]. Most of the effects caused by this amino acid are associated with its ability to increase the formation of NO, acting as a source for its formation [3]. It has been shown that the use of L-arginine reduces the size of the infarct, reduces vascular tone and causes a hypotensive effect, prevents and corrects ischemic and reperfusion damage to the brain and other organs [4].

An important role of ω-3 polyunsaturated fatty acids (Omega-3 PUFAs) is to ensure the functioning of cell membranes, transmembrane ion channels and the regulation of physiological processes through the synthesis of lipid mediators, which, lining up in the phospholipid layer of cell membranes, affect their fluidity [6]. Omega-3 PUFAs control the functioning of the immune and reproductive systems, being precursors for the biosynthesis of prostaglandins, leukotrienes and thromboxanes and other cytokines [5,6].

Brain neurons, being electrically active cells rich in ion channels, are the most sensitive to deficiency of polyunsaturated fatty acids [1,2,5,6].

Omega-3 PUFAs are involved in the implementation of the main functions of neurons, such as the transmission of impulses and the functioning of receptors [5,6].

In this regard, it is of interest to study the morphofunctional features of brain neurons in rats with subtotal cerebral ischemia against the background of the administration of Omega-3 PUFAs and L-NAME [4-6].

The aim of the work is to study morpho functional changes in rats with subtotal cerebral ischemia under conditions of using modulators of the L-arginine-NO pathway and against the background of the administration of Omega-3 polyunsaturated fatty acids.

Materials and methods of research

The experiments were carried out on 302 outbred rats.

The studies were conducted on animals represented by 8 groups of 6 rats each. The studies were carried out on animals represented by 8 groups of 6 rats each. The control group (group 1) consisted of sham-operated rats receiving 0.5 ml of isotonic NaCl solution. Subtotal cerebral ischemia (SCI) was modeled by ligation of both common carotid arteries (CCA) under conditions of intravenous thiopental anesthesia (40-50 mg/kg) - group 2. Rats of the 3rd group immediately before CCA ligation were injected intramuscularly with L-NAME at a dose of 5 mg/kg. Animals of the 4th group were additionally injected with L-arginine at a dose of 200 mg/kg of body weight (SCI + L-NAME + L-arginine), and rats of the 5th group received only L-arginine at a similar dose before surgery (SCI + L-arginine). Animals of the 6th group additionally received Omega-3 PUFAs intragastrically for a week at a dose of 5 mg/kg of body weight (SCI + L-NAME + Omega-3 PUFAs). Rats of the 7th group received only Omega-3 PUFAs in the same dose (SCI+Omega-3 PUFAs) before surgery. Rats of the 8th group were given L-NAME, L-arginine and Omega-3 PUFA in the above doses (SCI+L-NAME+L-arginine+Omega-3 PUFA) in combination. The control group consisted of sham-operated rats, which received 0.5 ml of isotonic NaCl solution.

The SCI duration was 60 minutes, after which the rats were decapitated.

Morpho functional changes in the cerebral cortex, tissue respiration of mitochondria of brain homogenates, indicators of oxidative stress and parameters of prooxidant-antioxidant balance, changes in stable metabolites of nitric oxide and platelet aggregation were studied in rats. The state of the endothelium was also assessed.

Morphological methods

For morphometric and histochemical studies of the cerebral cortex in CI (cerebral ischemia), after decapitation, the brain was quickly removed, pieces of

the anterior part of the cerebral cortex were fixed in Carnoy's fluid. Serial paraffin sections were stained with 0.1% toluidine blue by Nissl's method.

The study of histological preparations, their microphotography, morphometry and densitometry of the chromogen sediment in histological preparations were performed using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (LeicaDFC 320, Germany) and Image Warp image analysis program (Bitflow, USA). The localization of the parietal cortex and the hippocampal cortex in histological preparations of the rat brain was determined using a stereotaxic atlas. At least 30 neurons of the fifth layer of the parietal cortex and the pyramidal layer of the field CA1 of the hippocampus were evaluated in each animal. This provided a sufficient sample size for subsequent analysis.

To assess the severity of ischemic damage to the cerebral cortex, we studied changes in the size and shape of the perikaryons of neurons in the parietal cortex and hippocampus of rats, as well as the degree of staining of their cytoplasm (chromatophilia).

Result

Morphometry of neurons in the parietal cortex and hippocampus in the SCI group revealed a significant decrease in the area of their perikaryons - by 53% ($p<0.05$) and 49% ($p<0.05$), the elongation of neuron bodies increased by 20% ($p<0.05$) in each of the studied sections of the cerebral cortex, their roundness decreased by - 11% ($p<0.05$) and 22% ($p<0.05$), respectively (Table 1).

It is assumed that these changes in the size and shape of neurons are due to water-electrolyte disorders, as well as protein denaturation inside the cell.

In the groups like SCI + Omega-3, SCI + Omega-3 + L-NAME, SCI + L-arginine, SCI + L-NAME + L-arginine and SCI + L-NAME + L-arginine + Omega-3 there were no significant differences in compare with the indicators in the SCI group ($p>0.05$).

In animals of the SCI group, there was a decrease in the number of normochromic neurons and an increase in the number of hyperchromic neurons, as well as degenerative forms - hyperchromic shrunken neurons and shadow cells both in the parietal cortex and in the hippocampus (Table 2)

Groups of animals	**Areas of the cerebral cortex**	
	parietal cortex	**hippocampus**
	area, μm²	
Control	**145(130; 154)**	**109(100; 122)**
SCI	**69(67; 74) ***	**56(55; 57)***
SCI + Omega-3	**68(50; 84)***	**58(53; 84)***
SCI + L-NAME + Omega-3	**68 (54; 80)***	**57(40; 60)***
SCI + L-NAME + L-arginine + Omega-3	**69(64; 79) ***	**58(50; 73)***
	form factor, unit	
Control	**0,9(0,9; 0,9)**	**0,9(0,9; 0,9)**
SCI	**0,8(0,8; 0,8)***	**0,7(0,7; 0,8)***
SCI + Omega-3	**0,7(0,7; 0,8)***	**0,8(0,6; 0,8)***
SCI + L-NAME + Omega-3	**0,7(0,7; 0,8)***	**0,8(0,7; 0,8)***
SCI + L-NAME + L-arginine + Omega-3	**0,8(0,8; 0,8)***	**0,8(0,7; 0,8)***
	elongation factor, unit	
Control	**1,2(1,1; 1,3)**	**1,2(1,1; 1,3)**
SCI	**1,5(1,4; 1,5)***	**1,5(1,4; 1,6)***

SCI + Omega-3	**1,4(1,4; 1,5)***	**1,4(1,4; 1,4)***
SCI + L-NAME + Omega-3	**1,5(1,5; 1,5)***	**1,5(1,4; 1,6)***
SCI + L-NAME + L-arginine + Omega-3	**1,5(1,4; 1,5)***	**1,5(1,4; 1,6)***

Table 1 *- Sizes and shapes of perikaryons of neurons in the parietal cortex and hippocampus of control rats, with SCI, SCI + Omega-3 PUFA, SCI + L-NAME + Omega-3 PUFA and SCI + L-NAME + L-arginine + Omega-3, Me (LQ; UQ).*
Notes:
** - p<0.05 - in relation to the values in the "control" group*
- p<0.05 - in relation to the values in the "SCI" group
SCI - cerebral ischemia
L-NAME – Nω- nitro-L-arginine
Omega-3 - Omega-3 PUFA

Groups of animals	**Areas of the cerebral cortex**	
	parietal cortex	**parietal cortex**
normochromic neurons		
Control	**3208(3178; 3245)**	**3003(2989; 1945)**
SCI	**1932(1920; 1945)***	**2062(2009; 2298)***
SCI + Omega-3	**2143(1942; 2143)***	**2052(2001; 2167)***
SCI + L-NAME + Omega-3	**1942(1932; 2143)***	**2135(2001; 2269)***
SCI + L-NAME + L-arginine + Omega-3	**2066(1932; 2200)***	**2200(2066; 2269)***
hyperchromic neurons		
Control	**201(201; 268)**	**167(134; 201)**
SCI	**938(804; 938) ***	**737(670; 938)***
SCI + Omega-3	**1072(804; 1072)***	**1072(1072; 1140)$^{+}$**
SCI + L-NAME + Omega-3	**804(737; 1072)***	**804(804; 938)***
SCI + L-NAME + L-arginine + Omega-3	**938(804; 1072)***	**938(873; 1007)***
hyperchromic shrunken neurons		
Control	**134(67; 134)**	**134(0; 134)**
SCI	**670(670; 670)***	**670(670; 670)***
SCI + Omega-3	**603(536; 670)***	**536(536; 536)$^{+}$**
SCI + L-NAME + Omega-3	**670(536; 870) ***	**603(603; 672) ***
SCI + L-NAME + L-arginine + Omega-3	**670(536; 670) ***	**536(536; 672) ***
shadow cells		
Control	**134(0; 134)**	**134(134; 134)**
SCI	**404(269; 404)***	**402(269; 402)***
SCI + Omega-3	**269(269; 404)***	**134(134; 269)**
SCI + L-NAME + Omega-3	**404(404; 404)***	**335(269; 404)***
SCI + L-NAME + L-arginine + Omega-3	**404(404; 404)***	**338(269; 404)***

Table 2 *- The number of different forms of neurons per 1 mm^2 according to the degree of chromatophilia of the cytoplasm of the parietal cortex and hippocampus of rats of the control group, with SCI, SCI + Omega-3 PUFA, SCI + L-NAME + Omega-3 PUFA and SCI + L-NAME + L -arginine + Omega-3, Me (LQ; UQ).*

Notes:
** – p<0.05 – in relation to the values in the "control" group*
⁺ – p<0.05 – in relation to the values in the "SCI" group
 # – p<0.05 – in relation to the values in the group "SCI + Omega-3"
SCI – subtotal cerebral ischemia
L-NAME – Nω- nitro-L-arginine
Omega-3 - Omega-3 PUFA

In the SCI group in the parietal cortex, the number of hyperchromic neurons increased by 79% (p<0.05), hyperchromic shrunken cells - by 80% (p<0.05), shadow cells - by 67% (p<0.05). In the hippocampus, there was an increase in the number of hyperchromic neurons by 77% (p<0.05), hyperchromic shrunken cells - by 80% (p<0.05), shadow cells - by 67% (p<0.05), compared with indicators in the control group.

In animals of the SCI + Omega-3 group, compared with the control group, in the hippocampus, a decrease in the number of hyperchromic shrunken neurons by 75% (p<0.05) and an increase in the number of hyperchromic neurons (by 84%, p<0, 05). Compared with the SCI group, there was a decrease in the number of hyperchromic shrunken neurons by 20% (p<0.05) with an increase in the number of hyperchromic ones by 31% (p<0.05).

In the SCI + Omega-3 + L-NAME and SCI + L-NAME + L-arginine + Omega-3 groups, there were no significant differences compared to the SCI and SCI + L-NAME groups in the parietal cortex (p>0.05). Compared with the SCI + Omega-3 group, the number of hyperchromic neurons in the hippocampus was 25% less, hyperchromic shrunken neurons - 1% more (p<0.05), shadow cells - 60% (p<0.05).

Thus, subtotal cerebral ischemia leads to the development of morphofunctional disturbances of the cerebral cortex. The introduction of Omega-3 polyunsaturated fatty acids has a corrective effect on the hippocampus in conditions of subtotal ischemia, reducing the number of shadow cells and hyperchromic shrunken neurons, without significantly affecting the size and shape of neurons in the cerebral cortex. Prior administration of L-NAME, the use of Omega-3 did not prevent the effects of the NO synthase inhibitor and associated NO deficiency at this dose and route of administration.

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