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Research Article

Using L-Arginine-No Pathway Modulators in Rats with Subtotal Cerebral Ischemia. Histological Changes

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Abstract

Acute cerebrovascular accident is one of the most urgent problems in modern medicine. The frequency 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 origin tend to grow, rejuvenate, are associated with a severe clinical course, high rates of disability and mortality [1-6]. The relevance 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 morphological and functional disorders of the cerebral cortex. The introduction of a non-selective NO–synthase inhibitor - L-NAME aggravated histological disorders of neurons that occur with SCI: an increase in the number of hyperchromic shrunken neurons, a decrease in the size and deformation of their pericaryons. Additional use of L -arginine partially eliminated the negative effect of L – NAME [1-8].

Key words: cerebral ischemia; parietal cortex; hippocampus; neurons; rats

Introduction

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

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. 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.

Goal of the work - study of morphofunctional changes in rats with subtotal cerebral ischemia under conditions of using modulators of the L-arginine-NO pathway.

Materials and methods of research

Experiments were performed on 30 outbred rats.

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 intravenous thiopental anesthesia (40-50 mg/kg) - group 2. Rats of the 3rd group received intramuscular injections of L-NAME at a dose of 5 mg/kg immediately before CCA ligation, 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 the rats of the 5th group received only L-arginine in a similar dose (SCI + L-arginine) before surgery.

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

Morphofunctional changes in the cerebral cortex were studied in rats.

For a morphometric study of the cerebral cortex in BI 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 according to the Nissl 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 ImageWarp 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, which provided a sufficient sample size for subsequent analysis.

Research results

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).

Morphometry of neurons in the parietal cortex and hippocampus in the SCI group revealed a significant decrease in the area of their perikarya - 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).

Animal groups	Areas of the cerebral cortex	
	parietal cortex	hippocampus
	area, μm ^{2}	
Control	145(130; 154)	109(100; 122)
SCI	69(67; 74) *	56(55; 57) *
SCI + L-arginine	69(49; 84) *	57(53; 84) *
SCI + L-NAME	69 (59; 79) *	52(38; 58) *
SCI + L-NAME+L-arginine	67 (53; 81) *	56(41; 61) *
	form factor, units	
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 + L-arginine	0,8(0,8; 0,8) *	0,8(0,6; 0,8) *
SCI + L-NAME	0,7(0,6; 0,7) * $^+$	0,8(0,7; 0,8) *
SCI + L-NAME+L-arginine	0,8(0,8; 0,8) *	0,8(0,7; 0,8) *
	elongation factor, units	
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 + L-arginine	1,4(1,4; 1,5) *	1,4(1,4; 1,4) *
SCI + L-NAME	1,7(1,6; 1,8) * $^+$	1,7(1,6; 1,8) *
SCI + L-NAME+L-arginine	1,5(1,5; 1,5) *	1,5(1,4; 1,6) *

Table 1 - Sizes and shape of perikaryons of neurons of the parietal cortex and hippocampus of rats with cerebral ischemia, as well as isolated and combined administration of L-NAME and L - arginine, 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 - subtotal cerebral ischemia

L - NAME - Nω -nitro-L-arginine

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

In the SCI + L - NAME group, the neurons of the parietal cortex showed a decrease in the form factor - by 22% (p<0.05) compared with the values in the SCI group. Compared with the control group, in the parietal cortex, the area of neurons decreased by 52% (p<0.05), the form factor decreased by 22% (p<0.05), and the neuronal elongation factor increased by 29% (p <0.05). .05). No changes were found in the hippocampus, and compared with the indicators in the control group, there was a decrease in the area of perikaryal by 52%, the form factor - by 11% (p<0.05) and an increase in the elongation factor by 29% (p<0.05).

In the SCI+L-NAME+ L - arginine and SCI+ L -arginine groups, no significant differences were found in comparison with those 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 wrinkled neurons and shadow cells both in the parietal cortex and in the hippocampus (Table 2).

In the SCI group in the parietal cortex, the number of hyperchromic neurons increased by 79% (p<0.05), hyperchromic wrinkled 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 wrinkled 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 + L-arginine group, compared with the SCI group, there was a decrease in the number of hyperchromic shrunken neurons in the hippocampus by 75% (p<0.05) and an increase in the number of hyperchromic neurons (by 84%, p<0.05).

Animal groups	Areas of the cerebral cortex	
	parietal cortex	hippocampus
normochromic neurons		
Control	3208(3178; 3245)	3003(2989; 1945)
SCI	1932(1920; 1945) *	2062(2009; 2298) *
SCI + L- arginine	2143(1942; 2143) *	2052(2001; 2167) *
SCI +L-NAME	1928(1910; 1960) *	2075(2004; 2345) *
SCI + L-NAME+L- arginine	1942(1932; 2143) *	2135(2001; 2269) *
hyperchromic neurons		
Control	201(201; 268)	167(134; 201)
SCI	938(804; 938) *	737(670; 938) *
SCI + L- arginine	1072(804; 1072) *	938(938; 938) *
SCI +L-NAME	737(670; 737) *+	807(807; 874) *
SCI + L-NAME+L- arginine	804(737; 1072) *	804(804; 938) *
hyperchromic shriveled neurons		
Control	134(67; 134)	134(0; 134)
SCI	670(670; 670) *	670(670; 670) *
SCI + L- arginine	603(536; 670) *	536(536; 670) *
SCI +L-NAME	806(806; 806) *+	739(672; 807) *
SCI + L-NAME+L- arginine	670(536; 870) *	603(603; 672) *
shadow cells		
Control	134(0; 134)	134(134; 134)
SCI	404(269; 404) *	402(269; 402) *
SCI + L- arginine	269(269; 404) *	269(134; 402) *
SCI +L-NAME	404(269; 404) *	404(269; 404) *
SCI + L-NAME+L- arginine	404(404; 404) *	335(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 with cerebral ischemia, as well as isolated and combined administration of L-NAME and L - arginine, 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 - subtotal cerebral ischemia

L - NAME - Nω -nitro-L-arginine

In animals of the SCI+L-NAME group, there was a decrease in the number of hyperchromic neurons in the parietal cortex (by 22%, p<0.05) and an increase in the number of hyperchromic shrunken neurons (by 17%, p<0.05), compared with the SCI group, and compared with the control group, there was a decrease in the number of normochromic neurons by 40% (p<0.05), an increase in the number of hyperchromic neurons – by

73% ($p<0.05$), hyperchromic shrunken neurons – by 83% ($p<0.05$) and shadow cells – by 67% ($p<0.05$). In the hippocampus, no changes were detected compared to the "SCI" group ($p>0.05$), and compared to the "control" group, there was a decrease in the number of normochromic neurons by 31% ($p<0.05$) and an increase in the number of hyperchromic neurons - by 79% ($p<0.05$), hyperchromic shriveled cells - by 82% ($p<0.05$) and shadow cells - by 67% ($p<0.05$).

In the SCI + L - NAME + L -arginine group, no significant differences were found in the parietal cortex compared to the SCI and SCI + L - NAME groups ($p>0.05$).

Thus, subtotal cerebral ischemia leads to the development of morphological and functional disorders of the cerebral cortex. The introduction of a non-selective NO–synthase inhibitor - L-NAME aggravated histological disorders of neurons that occur with SCI: an increase in the number of hyperchromic shrunken neurons, a decrease in the size and deformation of their pericaryons. Additional use of L -arginine partially eliminated the negative effect of L - NAME.

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