

Amino Acid Pool in Rats with Brain Ischemia Attempt to Correct with Polyunsaturated Fatty Acids

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Received Date: June 09, 2023| Accepted Date: June 15, 2023| Published Date: June 29, 2023

Citation: E.I. Bon, N.Ye. Maksimovich, E.M. Doroshenko V.Y. Smirnov, E. Moroz, Pauliuchenkava D (2023), Amino Acid Pool in Rats with Brain Ischemia. Attempt to Correct with Polyunsaturated Fatty Acids, *Clinical Genetic Research*, 2(3); **Doi:**10.31579/2834-8532/027

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Abstract

In all types of ischemia, changes in the pool of amino acids were observed in both studied structures. In particular, an increase in the content of L-arginine was noted in patients with ischemia of varying severity, the most pronounced in ischemia (by 39%, $p<0.05$). Also, with all types of ischemia, there was an increase in glycine, while only with ischemia, an increase in the level of aspartate was noted, $p<0.05$. Along with changes in neurotransmitters, in the subtotal ischemia and partial ischemia groups, a decrease in the content of methionine was noted ($p<0.05$), while in total ischemia, on the contrary, an increase in the content of this amino acid was noted ($p<0.05$). In addition, with total ischemia, an increase in the content of threonine and tryptophan was noted. Changes in the pool of amino acids in rats with stepwise subtotal ischemia with intervals between arterial dressings on days 1 and 3 were the closest to changes in the subtotal ischemia group. So, in the 2nd sg with an interval between dressings of the artery for 3 days, the decrease in methionine and cysteine was the same as with subtotal ischemia, and there were no significant differences in the content of other amino acids. In the 3rd spg, with an interval between common carotid artery dressings of 1 day, as in subtotal ischemia, there was a decrease in the content of methionine, but, compared with subtotal ischemia, a decrease in cysteine was observed in parietal cortex ($p<0.05$). Changes in the pool of amino acids in the parietal lobe and hippocampus were of a similar nature, however, with stepwise subtotal ischemia, with an interval between dressings of both common carotid artery of 7 days, a decrease in cysteine occurred in the parietal cortex, and with partial ischemia, a more significant decrease in the level of methionine. The administration of Omega-3 polyunsaturated acids at a dose of 5 g/kg of body weight for a week to rats with SCI did not have a corrective effect on the levels of amino acids of taurine, methionine, L-arginine, and lysine, which changed during ischemia.

Keywords: amino acid; neurons; ischemia; omega-3 polyunsaturated acids

Introduction

Amino acids and their derivatives play an important role in the functioning of the brain, both in normal and pathological conditions, participating in the biosynthesis of membrane and signal protein and peptide molecules, some lipids, vitamins, hormones and biogenic amines, and are also directly involved in the implementation of neurotransmitter functions, regulation of the activity of excitation and inhibition processes (glutamate, aspartate, GABA) [10,11,12,21]. Aromatic amino acids (phenylalanine, tyrosine, tryptophan, histidine) are of particular importance as precursors of catecholamines and serotonin [3,6]. The study of the pool of amino acids is important for detailing the mechanisms of brain damage in cerebral ischemia [7,16,18,22].

The introduction of the drug Omega-3 acids "Omegamed" has a corrective effect on the structures of the hippocampus in conditions of subtotal cerebral ischemia, reducing the number of shadow cells and hyperchromic wrinkled neurons, while not affecting the size and shape of neurons in the parietal cortex of the brain and contributing to a lesser severity manifestation of neurological deficit. The study of the characteristics of the energy exchange of neurons in the brain of rats with subtotal cerebral ischemia against the background of the administration of Omega-3 acids showed a significant improvement in the parameters of mitochondrial respiration and an increase

in the content of ATP synthase in hippocampal neurons in animals with SCI [38,20,30,31,36].

In addition, against the background of the introduction of Omega-3 PUFA, correction of the parameters of antioxidant protection of brain neurons was noted, which was manifested by an increase in the content of total SH-groups of proteins and glutathione [2,39,32,33].

Methods

The study of the pool of amino acids was carried out in prepared homogenates of the studied structures of the brain of experimental animals, isolated 1 hour after ischemia modeling. To do this, after removing the brain, a fragment of the parietal cortex and hippocampus was taken, followed by freezing in liquid nitrogen.

Preparation of samples for the study included homogenization in a 10-fold volume of 0.2 M perchloric acid, centrifugation for 15 min. at 13000 g at 4°C, followed by collection of the supernatant. Amino acids were analyzed by reversed-phase chromatography with pre-column derivatization with o-phthalaldehyde and 3-mercaptopropionic acid in Na-borate buffer on an Agilent 1100 chromatograph [29].

To prevent a systematic measurement error, brain samples from the compared control and experimental groups of animals were studied under the same conditions.

Statistical processing. As a result of the research, quantitative continuous data were obtained. Since the experiment used small samples that had a non-normal distribution, the analysis was performed by nonparametric statistics using the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). The data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile.

When the applicability conditions were met (normality of the samples and homogeneity of the variances), parametric analysis of variance was used with a posteriori comparison of the selected contrasts.

Results

Changes in the pool of amino acids in rats with ischemic brain injury of varying severity were studied with partial (one-sided ligation of the common carotid artery, CCA), subtotal (simultaneous bilateral ligation of both CCAs), stepwise subtotal (alternate ligation of both CCAs at different time intervals) and total (complete cessation of cerebral blood flow) cerebral ischemia.

In partial cerebral ischemia (PCI), the following changes in the pool of amino acids (AA) were noted: an increase in the level of glutamate and GABA without changing the ratio of the content of excitatory and inhibitory amino acid transmitters, an increase in the content of L-arginine, a decrease in the level of essential AAs with an increase in the "Nonessential/Essential" AA ratio, as a reflection of the increased utilization of essential AAs. Changes in sulfur-containing AAs (cysteate, cystathionine, taurine, cysteine sulfinic acid) were absent, with the exception of a decrease in the content of methionine in the parietal lobe, which indicates minor violations of the prooxidant-oxidant balance in this model of CI [5,13,14,29]. There was a decrease in the content of branched hydrocarbon amino acids (BHAAAs) and a trend towards a decrease in the level of aromatic AAs (tyrosine, tryptophan, phenylalanine), with a decrease in their ratio, as a reflection of a more pronounced utilization of BHAAAs, compared with aromatic AAs. As shown earlier, PCI was accompanied by minimal changes in energy and prooxidant-antioxidant balance and a slight neurological deficit in terms of motor activity [30,31,33,34].

In subtotal cerebral ischemia (SCI), there was a decrease in the content of sulfur-containing AAs, with a decrease, in contrast to SCI, not only in methionine, but also in cysteate, as a reflection of a higher activity of oxidative stress [2,4]. Along with this, in SCI, as in PCI, an increase in the content of L-arginine, a tendency to an increase in the content of the inhibitory neurotransmitter glycine and a decrease in aspartate and glutamate as AA with the properties of excitatory neurotransmitters, as well as tryptophan, valine and leucine, were noted. At the same time, in contrast to PCI, there was no increase in the level of glutamate and a decrease in the levels of AA in the branched chain amino acids group. The revealed changes in the AA pool in the brain structures of rats with subtotal ischemia, modeled by simultaneous ligation of both CCAs, were accompanied by pronounced morphological changes in PL and Hp [34], high activity of oxidative stress [33], and the development of energy deficiency [35].

The introduction of Omega-3 fats at a dose of 5 g/kg of body weight for a week to rats with SCI did not have a corrective effect on the level of taurine, methionine, L-arginine and lysine, the change of which occurred in SCI; however, mythological studies revealed the presence of corrective properties on structural level [34], which is caused by the presence of vasoprotective and anti-inflammatory effects [27,28,38].

In rats with stepped cerebral ischemia (SSCI) with different intervals between ligation of both common carotid arteries (CCA), changes in the AA content were as follows.

In the SSCI subgroup with the longest interval between CCA dressings of 7 days (subgroup 1), there was a decrease in the content of BHAAAs - valine and leucine, an increase in the content of tryptophan, methionine, L-arginine and glycine. As in the case of PCI, in rats of the 1st subgroup of SSCI, there

was an increase in the content of L-arginine, a decrease in the content of amino acids with a branched hydrocarbon chain, however, in contrast to PCI, there was an increase in the content of valine, leucine and tryptophan, but there was no change in the content of sulfur-containing compounds. In PCI and SCI there were a decrease in the level of methionine. When compared with SCI, in rats of the 1st subgroup of SSCI, as in SCI, there was an increase in glycine ($p < 0.05$), however, in contrast to SCI, there was a decrease in the content of cysteate and methionine ($p < 0.05$).

In the subgroup SSCI with an interval between CCA dressings of 3 days (subgroup 2), as in SSCI with an interval between CCA dressings of 7 days, PCI and SCI, there was an increase in the content of L-arginine, a decrease in the content of methionine [25]. However, in contrast to the 1st subgroup of SSCI and from other groups of CISS (PCI and SCI), in the 2nd subgroup of SSCI there was an increase in the content of taurine and nonessential AA - asparagine and alanine. As with PCI, there was an increase in the content of glutamate, and, as with SCI, a decrease in the level of cysteate, but, compared with PCI, there was a decrease in the content of asparagine and an increase in the level of alanine [9,17,19].

Changes in the 3rd subgroup of SSCI with the shortest interval between CCA dressings - 1 day (subgroup 3) manifested themselves as an increase in the content of tyrosine, L-arginine and citrulline, taurine, asparagine and alanine, as well as a decrease in the content of ornithine and methionine [15]. Compared with the 1st subgroup of SSCI, in this subgroup there was a decrease in the content of methionine and lysine, as well as an increase in the content of asparagine, alanine, L-arginine, ornithine and citrulline. Compared to the 2nd subgroup of SSCI, there was a significant increase in the level of asparagine, lysine, tyrosine, arginine and citrulline. The nature of the shifts in the AA pool in the 2nd and 3rd subgroups of SSCI was of the same type, with the exception of an increase in the level of citrulline in the 3rd subgroup, which indicates a more significant decrease in the synthesis of nitric oxide in this subgroup of SSCI. In the 3rd subgroup of SSCI, similarly to the group "PCI", there was a decrease in the content of methionine, but an increase in the content of a number of AAs - asparagine, alanine, lysine and tyrosine was observed [20]. In addition, similarly to the SCI group, there was a decrease in the content of methionine, but, compared with SCI, a decrease in the content of cysteate was observed in the PL [1].

When comparing the changes in SSCI in 3 subgroups with different intervals between CCA ligations, a similar pattern of changes in the AA pool was revealed, which manifested itself in an increase in the content of L-arginine, which was also noted in rats with PCI and SCI [24]. However, changes in other AAs in the 3rd subgroup, SSCI with a minimum interval between ligation of both common carotid arteries, 1 day was the most pronounced and included an increase in the content of tyrosine, citrulline, taurine, asparagine and alanine, as well as a decrease in the content of ornithine and methionine, as a reflection of the ineffectiveness of compensatory mechanisms in this model of CI.

Changes in the pool of amino acids in the parietal lobe and hippocampus were framed along the same lines, however, with SSCI with an interval between ligation of both common carotid arteries of 7 days, a decrease in cysteate occurred in the PL, and a more significant decrease in the level of methionine than in HP [26].

In previous studies, it was found that SSCI with an interval between CCA dressings of 7 days is manifested by a slight difference in the size of neuronal perikaryons and the ratio of neurons in terms of the degree of cytoplasmic chromatophilia, energy exchange indicators and prooxidant-antioxidant balance from those in the group with PCI, which can be explained by the inclusion of compensatory mechanisms that prevented the development of significant disorders [32]. SSCI with an interval of 1 and 3 days between dressings of both CCAs leads to damage to neurons, which manifests itself in a decrease in their size, deformation of perikaryons, an increase in the number of shriveled neurons and shadow cells, a significant disorganization of neuronal organelles, inhibition of energy exchange and activation of oxidative stress [30,31,33].

In total cerebral ischemia (TCI), the studied brain structures showed an increase in the content of aromatic AAs of tyrosine and tryptophan. The

content of methionine also increased, in contrast to SCI and PCI, in which the content of methionine decreased, which may be due to the lack of activation of oxidative processes in this form of cerebral ischemia. Along with this, with TCI, as with SCI, PCI and all types of SSCI, an increase in the content of L-arginine was noted, a tendency to an increase in the content of the inhibitory neurotransmitter glycine [8,23,36].

Also, when modeling TCI, the most pronounced morphofunctional disorders were revealed: shriveled and disturbances in the ultrastructure of neurons, depression of mitochondrial respiration, and suppression of antioxidant protection [31,33,34,37].

Compared with the indicators in the control group, in rats with SCI after the introduction of Omega-3 PUFAs, an increase in the content of L-arginine by 14% in PL ($p < 0.05$) and by 36% in HP ($p < 0.05$), alanine - by 38% in PL ($p < 0.05$) and by 36% - in HP ($p < 0.05$) and a decrease in the content of valine as a branched hydrocarbon amino acid (BHAA) by 6% - in PL ($p < 0.05$) and by 31% in HP ($p < 0.05$). Also, the content of lysine remained less than in the control by 50% - in PL ($p < 0.05$) and by 43% - in HP ($p < 0.05$), and the sulfur-containing AA "methionine" - by 19% ($p < 0.05$) and by 26%, respectively ($p < 0.05$) [7,10,19, 22,23,29].

At the same time, compared with the SCI group, after the introduction of Omega-3 PUFAs, there were no differences in the content of AA ($p > 0.05$) [11,12,13,27,28].

Thus, the administration of Omega-3 PUFAs at a dose of 5 g/kg of body weight for a week to rats with SCI did not have a corrective effect on the levels of AA of taurine, methionine, L-arginine, and lysine, which changed during SCI.

The amount of research carried out fully reveals the tasks set for studying changes in the pool of amino acids in rats with cerebral ischemia of varying severity.

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