BIOCHEMICAL MARKERS OF BRAIN DAMAGE IN ACUTE ISCHEMIC STROKE

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This article showed the investigation of the L-arginine and nitric oxide products levels in blood serum and liquor in acute ischemic stroke. As against the control in ischemic stroke on the 1st and 3rd days was observed significant decrease of L-arginine and nitric oxide products levels in liquor dependently on severity of the disease, whereas in blood serum were observed opposite changes.

Keywords: ischemic stroke, liquor, blood serum, L-arginine, nitric oxide

Stroke is a major healthcare problem afflicting more than 40 000 individuals and the second or first leading cause of death in Uzbekistan (Gafurov B.G, 2009; Asadullaev M.M., 2004). In the last decades substantial research and efforts have been made to understand the biochemical mechanisms involved in brain damage and to develop new treatments.

The progress of understanding the pathogenesis of neurovascular pathology now is connected with the study of role of neuroactive amino acids and a universal regulator of cellular and tissue metabolism – nitric oxide (NO). Interest in NO chemistry soared after recognition in 1987 of the biological role of NO, and NO has recently emerged as a possible key mediator of oxidative stress and disease. In medicine NO is applied as a drug for ischemic heart attacks and hypertonic crises, rendering vasodilatatory action. NO is proved adjusts vascular tonus, inhibits thrombocytes aggregation and their adhesion to a cellular membrane, functions in central and vegetative nervous system, has cytotoxic action, participating in antimicrobial, antiviral and antitumor immunity [1-8].

Because L-arginine (L-arg) is the only known substrate for nitric oxide (NO) generation, the purpose of our investigation was to study L-arg and NO levels in blood serum and liquor after cerebral ischemia and connection between their levels and stroke gravity.

Material and methods. 58 patients with acute ischemic stroke (AIS) at the age of 35-80 years (average age – 61,36 ± 0,19 years) were investigated (basic group). Male were 30 (51,7 %), female – 28 (48,3 %). Control group included 24 persons without stroke with similar age and gender. All patients with AIS were admitted to the clinic within the first day, the basic part of them (57,02 %) was admitted at the first 6 hours from the beginning of disease, and at the period from 7 to 12 hours – 29 % of the patients. For evaluation of severity degree of patients' condition and expressed neurological defect, on admission period and in dynamics of AIS the clinical symptoms, dynamics of restoration of neurological functions were estimated on basis of Scandinavian stroke scale (SSS). For biochemical studies were taken blood from ulnar vein and liquor by lumbar puncture in both groups (after agreement with patients and for diagnostic purpose). NO level was determined with using Grees reactant (1 % of sulfanilamide, 0,1 % naphtylindiamid, 2,5 % phosphoric acid) on spectrophotometer by allocation NO derivatives (NO_2, NO_3)

[3, 4, 6] and L-arg level by high performance liquid chromatography method [9] on chromatograph Du-Pont 8800, column 250/8/4 Nucliosil 100-5 C18. All reagents made by «Sigma», USA.

Results and discussion. On the basis of estimation of clinical signs, dynamics of restoration of neurological functions and SSS, all AIS patients were divided into four groups. The first group included 5 patients (8,6 %) with mild AIS (total clinical score in SSS was – 44,4 ± 1,3). Second group included 25 patients (43,1 %) with average degree of severity (total clinical score – 24,2 ± 0,9). The third group consisted of 22 patients (37,9 %) with severe AIS (total clinical score – 10,3 ± 0,93). In the fourth group were 6 patients (10,3 %) with extremely severe course of AIS (total clinical score – 6,2 ± 0,8).

We investigated the L-arg and NO levels in liqour and blood serum in all patients. Per the first day of AIS onset, liquor L-arg and NO levels authentically decreased 3,87 (142,5 ± 71,4 vs. 551,5 ± 72,6 nmol/ml) and 1,06 times (0,848 ± 0,021 vs. 0,903 ± 0,041 mmol/l, $P<0,05$) relatively after the ischemic event. Serum L-arg and NO levels increased 2,01 (1068,9 ± 36,9 vs. 531,8 ± 45,2 nmol/ml) and 1,02 (0,922 ± 0,057 mmol/ml vs. 0,909 ± 0,037 mmol/l) times relatively to comparison with control, peaked between 6 and 24 hours (table).

Table

The L-arg and NO levels in liquor and blood serum in different groups of the patients (M ± м)

Group of patients	L-arg (nmol/ml)		NO (mmol/ml)	
	Liquor	Blood serum	Liquor	Blood serum
Basic group (AIS):	142,5 ± 71,4*	1068,9 ± 36,9	0,848 ± 0,021*	0,922 ± 0,057
1st group	235,5 ± 68,4	905,7 ± 42,5*	0,979 ± 0,027	0,855 ± 0,027
2nd group	141,2 ± 72,6**	1005,1 ± 42,1**	0,816 ± 0,037**	0,875 ± 0,04**
3rd group	136,8 ± 66,5**	1023,5 ± 38,6**	0,491 ± 0,23**	0,903 ± 0,021**
4th group	102,6 ± 56,2	1058,2 ± 29,3**	0,430 ± 0,211	0,979 ± 0,037**
Control group	551,5 ± 72,6	531,8 ± 45,2	0,903 ± 0,041	0,909 ± 0,037

N o t e : * – reliability of distinctions with control group ($P < 0,05$).
** – reliability of distinctions between groups of the patients with different severity degree of AIS ($P < 0,05$).

The comparison of the study has shown that the L-arg and NO levels essentially does not depend on localization of the ischemic locus on basis of the clinical data, CT/MRI. However, depending on severity of AIS, liquor L-arg and NO levels were significantly lower in patients with severe and extremely severe state, in those with poor outcome. There was a negative correlation in blood serum (table). In dynamics of disease L-arg and NO metabolites levels at AIS decreased by the 3rd day 1,89 and 1,3 times, by the 10th day 3,87 and 1,99 times relatively.

Thus, the study showed that L-arg-NO system exposed considerable changed in stroke and depended on its severity. The distinctions of metabolic shifts in liquor

and blood serum are explained to that on the L-arg and NO levels in blood serum render influence a condition of metabolic processes not only in a brain, but also in other organs and tissues. The L-arg-NO system in liquor was lower, the more severe AIS, resulting to irreversible consequences and mortal outcome. However, as the NO synthesis depends on quantity of excitatory amino acid glutamate, and it remained in liquor authentically increased [5, 7], infringement of NO synthesis at AIS, probably, has other mechanism. Probably, failure of NO synthesis at AIS is caused by decrease of quantity of its substrate – L-arg for NOS, acceleration of its metabolism or their combination. The activation of L-arg-NO system in liquor has, probably, compensator character. The evidence suggests that NO can exert both protective and deleterious effects depending on factors such as the NOS isoform and the cell type by which NO is produced or the temporal stage after the onset of cerebral ischemia. Immediately after brain ischemia, NO releases from eNOS and renders protective action mainly by promoting vasodilatation; however, at the subsequent stages NO produced by overactivation of nNOS and, later, by de novo expression of iNOS, participating in the brain damage [8].

Taken together we can do some conclusions:

1. The reduction of the Arg and NO levels in liquor can play a role in the forecast of AIS. It is possible, that some clinical symptoms are connected with «excitotoxicity» phenomenon. NO, probably, can play a double role at AIS, showing at the beginning of disease neuroprotective, and in later stages neurotoxic action.

2. The study of the L-arg-NO – system role represents perspective scientifically proved direction capable to supply progress in clinical neurology. Determination of L-arg levels in blood and liquor might be helpful to evaluate NO compensator or neurotoxic action.

3. The exact role of these and other neurotransmitters in the pathogenesis of neuronal injury observed in acute stroke needs to be defined.

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