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The fluctuation of free amino acids in serum during acute ischemic stroke

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ABSTRACT

Currently, little data exists regarding the involvement of free amino acids (AA) in the pathogenesis of ischemic stroke (IS). Thus, our objective was to study the degree of the degree of fluctuation of free amino acids level in serum during the acute phase of IS. The study consisted of eighteen patients (female/male: 10/8; age: 73.1 ± 4.1) with acute IS that was confirmed by way of computed tomography, while twelve sex and age matched individuals were assigned as control group. During the study period, the patients did not receive any supplemental amino acids therapy that could affect the obtained results. The venous blood was obtained after >3 hours fasting at two time-points; time-point 1 – at admission to the hospital; time-point 2 – on day 5 from stroke onset. The blood for control purposes was collected only once, and the blood collection at time-point 1 was done before thrombolytic treatment (nine patients). The amino acids were identified using the Amino Acids Analyser (AAA 400) by INGOS Corp., Praha, Czech Republic. Our results revealed a statistically significant increase of glutamate, cystine and methionine on day 1 of stroke, in comparison to control, whereas, proline level was decreased on day 1 of stroke – in comparison to control serum. On comparing day 5 to the initial day of IS, elevation was observed of levels of asparagine, glycine, tyrosine, arginine, threonine, valine, leucine and phenylalanine. It can be said, then, that ischemic stroke induces both essential and nonessential amino acid fluctuations. Moreover, the decrease in proline and glutamine serum level with the simultaneous increase in the concentration of branch chain amino acids, Glu and Thr suggests a violent mobilization of the body's proteins. Thus, a decrease of Pro and a simultaneous increase of Glu serum level could be considered as a marker of acute IS.

INTRODUCTION

Worldwide, ischemic stroke (IS) is one of the leading causes of death and disability [8]. Contrary to other diseases, e.g. heart failure, the use of biomarkers for stroke detection is not accepted for routine application [14], and only head imaging, together with neurological assessment, gives a reliable diagnosis of stroke [10]. However, research of the fluctuation of various biochemical agents during acute IS (AIS) is needed for two main purposes: first, to help understand the biochemical changes that occur during the stroke, and, secondly, to discover a sensitive and specific indicator similar to brain imaging that can be used in clinical practice for diagnosis of stroke.

Currently, little data exists regarding fluctuation of free amino acids (AA) and their involvement in the pathogenesis of IS. Still, it is known that the plasma levels of excitatory AA such as glutamate (Glu) increase during AIS. Moreover, the Glu serum level measured within the first 6 hours of IS positively correlates with the ischemic focus volume [2]. However, thrombolytic therapy applied for AIS treatment has led to an early decrease in the Glu level, simultaneously with an increase in the level of neuroprotective γ-aminobutyric acid (GABA) [9]. Furthermore, the study performed by Kimberly *et al.* reveals a decrease in branched chain amino acids (BCAA), leucine (Leu), isoleucine (Ile),

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and valine (Val) after large infarction due to cardioembolic stroke [7]. In addition, one recent study in Japan has demonstrated that a dietary intake of glutamic acid and glycine may be associated with a higher risk of stroke mortality. This suggests they play a role in the IS pathogenesis [11]. However, currently there is no reliable evidence suggesting the possibility of using AA as the markers of stroke. Our objective, therefore, was to study the fluctuations of the free amino acid level in the serum of patients during the acute phase of IS.

MATERIAL AND METHODS

Patients

Eighteen patients with acute IS that was confirmed using the computed tomography (CT) were enrolled. Twelve sex and age matched individuals were assigned as control group. For IS patients, their neurological status was evaluated at admission via the use of the *National Institutes of Health Stroke Scale* (NIHSS). Nine of the study's IS patients were enrolled to *iv.* thrombolytic therapy with Actylise (Boehringer Ingelheim International, Ingelheim,Germany). During the study period, the patients did not receive supplemental amino acids therapy that could affect the obtained results. The characteristics of the study patients is given in Table 1. The local Ethics Committee (Medical University of Lublin) accepted the protocol of the study (agreement No. KE-0254/260/2013).

Table 1. Characteristic of study group

	Stroke patients n=18	Control n=12	X^2
Age (mean ± SD)	73.1±4.1	69.2±3.2	$p>0.05$
Sex (male/female)	10/8	6/6	
Hypertension (yes/no)	13/5	4/8	
Diabetes (yes/no)	3/15	0/12	
Coronary dis. (yes/no)	13/5	2/10	
Atrial fibrillation (yes/no)	5/13	1/11	
Smoking (yes/no)	11/7	7/5	
OCSP classification TACI PACI LACI POCI	 1 9 6 2		
NIHSS at the admission (median, 1st-3rd quartile)	12 (7-19)		
NIHSS at the discharge (median, 1st-3rd quartile)	3 (1-15.5)		

OCSP (*Oxford Community Stroke Project)* classification, TACI (*total anterior circulation infarct*), PACI (*partial anterior circulation infarct*), LACI (*lacunar infarct*), POCI (*posterior circulation infarct*), NIHSS (*National Institutes of Health Stroke Scale*)

Sample collection

The venous blood was obtained after >3 hours fasting at two time-points; time-point 1 – at their admission to the hospital; time-point 2 – on day 5 from stroke onset. The blood used for Control was collected only once. Time-point 1 covered the first 5±4 hours of stroke (from "last seen well"). Because free arginine is the constituent of Actylise, the blood collection at time-point 1 was done before the application of thrombolytic treatment.

AA analysis

For amino acids analysis, serum samples were deproteinised with 6% sulphosalicylic acid in lithium-citrates buffer (pH 2.8), and centrifuged. AA were determined by way of automated ion-exchange chromatography, utilizing five lithium-citrate buffers, via the Amino Acids Analyser (AAA 400) by INGOS Corp., Praha, Czech Republic. The individual amino acids were separated using analytic column OSTION LG FA. The amino acids were identified through comparison to the standards provided by INGOS Corp. The original software MIKRO version 1.8.0 (INGOS, Praha, Czech Rep.) was employed for AA evaluation, and the amino acids serum level was expressed in μM/ml.

Statistical analysis

The presence of Gaussian distribution was estimated by way of the Lillefors test. The repeated measures analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons post-hoc test or t-test were used for statistical analysis. A p-value < 0.05 was considered statistically significant.

RESULTS

The fluctuation of essential and nonessential amino acids during the acute phase of IS is presented in Table 2. A statistically significant increase in Glu, cystine (cysteine is rapidly oxidized under physiological pH to cystine (Cyss)), and methionine (Met) was noticed on day 1 of stroke, in comparison to the control, whereas the proline (Pro) level

Table 2. The fluctuation of free amino acids in serum of stroke patients. CYSS – cystine; ANOVA with Tukey-Kramer post hoc test; arrows indicate the increase or decrease of amino acid serum level *vs.* control or day 1 respectively

Non-essential amino acids					
AA	Control	Day 1	*vs* Control	Day 5	*vs* Day 1
ASN	0.03586± 0.01	0.03456± 0.02256		0.05225± 0.0276	↑*
GLN	0.5742± 0.1444	0.4575± 0.1934		0.3986± 0.1524	↓(*)
GLU	0.05986± 0.0324	0.1341± 0.058	↑↑**	0.1458± 0.065	↑(*)
GLY	0.2642± 0.1049	0.2492± 0.08395		0.304± 0.08395	↑↑**
TYR	0.05514± 0.01985	0.06869± 0.01985		0.08456± 0.02068	↑*
ARG	0.1202± 0.02445	0.1268± 0.01356		0.15167± 0.02347	↑*
ASP	0.042± 0.00858	0.04314± 0.0209		0.05338± 0.01246	
SER	0.12243± 0.01921	0.1275± 0.03586		0.1458± 0.02621	
PRO	0.62314± 0.1811	0.27413± 0.1502	↓↓↓***	0.3198± 0.17959	
ALA	0.468± 0.06959	0.3726± 0.10336		0.40594± 0.08693	
CYSS	0.00043± 0.0007	0.00656± 0.01268	↑*	0.01181± 0.01434	
HIS	0.06971± 0.01137	0.07919± 0.01684		0.08± 0.0171	
Essential amino acids					
AA	Control	Day 1	*vs* Control	Day 5	*vs* Day 1
THR	0.13357± 0.05247	0.10275± 0.01941		0.1574± 0.03667	↑↑↑***
VAL	0.1814± 0.03787	0.22069± 0.056		0.29188± 0.07806	↑↑**
LEU	0.097± 0.0224	0.1295± 0.05241		0.1745± 0.06735	↑*
PHE	0.04571± 0.0077	0.0625± 0.01394		0.08181± 0.02047	↑↑**
MET	0.01429± 0.00854	0.02788± 0.01518	↑*	0.02956± 0.01316	
ILE	0.04071± 0.01426	0.0535± 0.02645		0.06963± 0.03408	
TRP	0.06314± 0.01549	0.06506± 0.02946		0.07444± 0.02404	
LYS	0.1701± 0.037	0.26156± 0.1599		0.23869± 0.05152	

(*) p<0.1; * p<0.05; ** p<0.01; *** p<0.001

was decreased on day 1 of stroke, in comparison to the control serum. On comparing day 5 to the initial day of IS, elevation of the asparagine (Asn), glycine (Gly), tyrosine (Tyr), arginine (Arg), threonine (Thr), Val, Leu and phenylalanine (Phe) levels was observed. Still, the increase in Glu and the decrease in glutamine (Gln) serum levels did not reach statistical significance ($p < 0.1$).

DISCUSSION

Besides their role in peptide and protein synthesis, free AA are precursors of several biologically active compounds (e.g. taurine, heme, nitrogenous bases) and hormones (epinephrine, norepinephrine, dopamine, thyroid hormones) [23]. In addition, AA can regulate gene expression at transcriptional [1], translational, and post-translational levels [5].

Furthermore, an elevated AA level (e.g. after intravenous administration) can stimulate the secretion of hormones from the endocrine cells [12] and affect hormonal homeostasis. What is more, after the administration of Arg, secretions of insulin, growth hormone, prolactin, glucagon and progesterone are increased [24].

The AA plasma level is relatively constant in healthy adults. However, in several diseases, circulating AA undergo dynamic changes [23], yet, data relating to the fluctuation of the amino acid level in serum during IS are elusive. A previous study has revealed a decrease in BCAA (which are essential amino acids) in the course of acute cardioembolic stroke [7]. Moreover, our preceding study revealed an early decrease in the BCAA plasma level measured in patients after myocardial infarction (MI). In this study, the BCAA level was restored to the values noticed in control patients on day 5 after MI [18]. Still, it was recognized that the serum level of BCAA in patients with stable coronary heart disease (CHD) is decreased in comparison to healthy controls [18]. Furthermore, a current study has shown that Leu, Val, and Ile measured at time-point 1 did not significantly differ in comparison to the control; however, the Leu and Val level was elevated on day 5, compared to time-point 1. The elevation of plasma BCAA was previously noticed in individuals after 3 days of fasting, in parallel with increased protein degradation [20]. Therefore, the observed rise in the BCAA level either can indicate the nutritional state of stroke patients or can be the cause of intense metabolic processes due to increased activity of proinflammatory cytokines. In addition, the elevation of the Phe and Tyr serum level after stroke can be generated by the competitive transport of BCAA and aromatic AA into the central nervous system, subsequently leading to disturbances in the synthesis of catecholamines thereafter [21].

In our work, a significant 2-fold decrease in Pro and a 2-fold increase in Glu were observed on day 1, in comparison to the control samples. This suggests that changes in Pro and Glu could be considered as a marker of AIS in future research. As mentioned above, it is known that Glu increases early in plasma during AIS [2]. Pro plays an important role in the stimulation of glutaminergic neurons, and Pro has been proposed as being a neuromodulator and neurotransmitter in the central nervous system [19]. It is synthesized mostly from Gln *in vivo* [6]; therefore, the observed decrease in Pro could have been brought about by the Gln decrease (insignificant in our research). Pro deficiency is known to impair collagen synthesis [13], which may be significant during the ischemia repairing processes. What is more, former research has shown that the serum level of a majority of the analyzed amino acids, except Gln ($p<0.1$), rises in the progress of stroke. In our work, the trend towards the decrease in Gln on day 5 of stroke could have been induced by its degradation to Glu and ammonia, resulting in an increase in the Glu serum level. In addition, the observed elevation of Glu in IS patients *vs*. controls could have been caused by its release from damaged neurons, leading to excitotoxicity – an important factor for the neuronal cell death during brain ischemia [9]. However, the higher Glu serum level (after iv. AA administration) was seen to result in an increase in insulin secretion [12,24], which could have reduced hyperglycemia and might potentially improve the stroke outcome [17].

It should be noted that half of the study patients received thrombolytic therapy with rtPA. To improve the solubility of rtPA, L-arginine is added into the drug as its constituent, and patients during thrombolysis received approximately 3.0 g of L-arginine [3]. The previous study revealed an increase in the Arg serum level directly after the thrombolysis, but on day 5 of stroke, the Arg level was restored to the level noticed in IS patients without thrombolytic therapy [4].

AA are generally stable at the physiological solution. However, cysteine is rapidly oxidized to cystine [23]. Therefore, cystine was found in the serum of both the study and control patients. The mean cystine level was dramatically (but statistically insignificantly) increased in the serum on day 5 of stroke. However, the increase in Cyss on day 1 *vs*. controls is consistent with other research [15]. Cysteine is known to promote neuronal cell death and was reported to be elevated in brain ischemia. Raised cystine (and cysteine) in the serum of patients with ischemic stroke may, therefore, indicate increased production of H_2S in the brain, resulting in poor outcome [22]. The experimental study showed that a methionine metabolite homocysteine induces the death of glial cells. This observation suggests involvement of homocysteine in neurodegeneration [16].

CONCLUSIONS

Ischemic stroke brings about both essential and nonessential amino acid fluctuations. This suggests the violent mobilization of the body's proteins. Furthermore, the decrease of Pro and increase of Glu serum level could be considered as being a marker of acute IS.

CONFLICT OF INTERESTS

The authors do not declare a conflict of interests.

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