

Relation of several indexes of endogenous protection system with neurological outcome on 14th day of ischemic stroke

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ABSTRACT

Study purposed to evaluate several indexes of protection system in acute and sub-acute stages of ischemic stroke and to establish their relation with neurological outcome at 14th day. 95 patients aged 45 to 75 years, 54 female and 41 male investigated. Severity of disease evaluated by international scales GCS and NIHSS. Patients divided into three groups: 1st (27 patients) – with severe stroke (GCS>9, NIHSS>15), 2nd (39 patients) – with moderate severity (GCS=14-15; NIHSS=10-15), 3rd (29 patients) – with mild stroke (GCS=15, NIHSS<15). Again, all patients evaluated by NIHSS on 14th day of stroke. In 48 hours of symptoms onset and on 14th day blood levels of interleukine-10 (IL-10) detected by enzyme-linked immunosorbent assay and blood superoxidismutase levels (SOD) detected by spectrophotometric method. Results were processed by SPSS statistical computer packet. ANOVA analysis and multivariate logistic regression were used. IL-10 and SOD levels found significantly elevated on 14th day against initial indexes and control ($p<0,05$). 1st group revealed the decreased levels of IL-10 and SOD compared to 2nd and 3rd groups ($p<0,05$). Significant negative correlation found between IL-10 and SOD levels and NIHSS score at 14th day ($r=-0,28$ $p<0,05$ and $r=-0,31$ $Sp<0,05$ respectively). Apparently, stroke outcome significantly depends on activation of internal bio-protective system.

KEYWORDS: *interleukines, inflammation, penumbra, ischemia, protection*

As it is known, the ultimate results of cellular, biochemical and metabolic consequences of acute ischemic stroke, which lead to irreversible neuronal injury greatly depend on the activity of endogenous bio-protection system. The mentioned system comprises enzymes-antioxidants, free radical scavengers, growth factors, anti-inflammatory cytokines, insulin-like factors and etc. Their actions are directed toward different targets but in complex they all influence the survival of brain tissue by inhibiting the signaling cascades of necrosis and apoptosis. The effect of growth factors is mediated by activation of series of kinases that translocate to the nucleus and phosphorylate transcriptional factors inducing the cells to grow, differentiate or to obtain enough trophic support to survive [13]. Anti-inflammatory cytokines (IL-4, IL-10) suppress the complex network of pro-inflammatory agents, thus blocking the expression of intracellular adhesion molecules (ICAM-1, VCAM-1) and matrix metalloproteinases in neurovascular unit of penumbra region [3,6,12]. They decrease the migration of leukocytes through damaged blood-brain barrier, modulate the neuronal sensitivity to excitotoxicity, inhibit the inducible NO-synthase [9]. Enzymes anti-oxidants inhibit the initiation of free radicals by scavenging them and stabilizing the level of oxidative/peroxidative reactions leading to the inhibition of mitochondrial respiratory chain proteins, disruption of cellular membranes, blood-brain barrier, DNA, damage of neurons and endothelial cells [8]. During acute phase of stroke the endogenous protection system is strongly suppressed, but according to genetically determined mechanisms in time dependant manner it regains its strength and resists to the adverse effects of brain ischemia. It is suggested that the expression of protective genes is influenced not only by severity of ischemia but also by several risk factors, which can have the real impact on the activity of protection systems [2].

The present research is aimed at investigation of several indexes of endogenous protective system in acute and sub-acute stage of ischemic stroke and their relation with neurological outcome at 14th day of stroke and with risk-factors of stroke as well.

MATERIALS AND METHODS

Research involved 95 ischemic stroke patients aged 45 to 75 years, 54 female and 41 male admitted at neurological clinic of Georgian State Medical Academy during 2000-2004. Exclusion criteria comprised acute inflammatory and autoimmune disorders, severe somatic pathology, and coma. Control groups has been consisted with 25 age-matched healthy persons, who did not reveal any significant signs of cerebrovascular pathology. Etiology of stroke classified according to TOAST criteria [1]. Several non-modifiable and modifiable risk factors of stroke were studied retrospectively (age, sex, inheritance, history of TIA or previous stroke, hypertension, atherosclerosis, atrial fibrillation, diabetes mellitus, smoking, alcohol abuse, acute infections 1-2 months before stroke, psychological stress). Blood flow in extra- and intracranial arteries were evaluated by duplex-scanning (HDI Ultramark 9-linear multi-frequent transducer 7-11 MHz) and by transcranial dopplerography (DWL Multi-Dop T with pulse-wave transducer 2 MHz). Data from duplex-scanning and high blood cholesterol levels were used to establish atherosclerosis as a risk-factor. Severity of stroke in 48 hours after the onset of disease and neurological outcome at 14th day was evaluated by international scales: Glasgow Coma Scale (GCS) and National Institute Health Stroke Scale (NIHSS).

Patients were divided into three groups: 1st group- 27 patients with severe stroke (GCS>9, NIHSS>15), 2nd group – 39 patients with stroke of moderate severity (GCS=14,15; NIHSS=10-15) and 3rd group - 29 patients with mild stroke (GCS=15, NIHSS<15). On 14th day from disease onset decrease of NIHSS score at least 1 point was considered as amelioration, while the increase of NIHSS score at least 1 point was considered as deterioration. Treatment was conducted according to internationally recognized evidence-based guidelines. Anticoagulants were administered only in the cases of cardio embolism, when neuroimaging and clinical examination excluded the large cerebral lesions.

For special laboratory investigations 7 ml blood was taken within first 48 hours and on 14th day of admission from patients and only one-time from controls. Blood

samples were centrifuged on 1000g and plasma was frozen and preserved at - 20°C for further assay.

Blood levels of anti-inflammatory cytokine interleukine-10 (IL-10) were detected by enzyme-linked immunosorbent assay (ELISA) by application of ELISA- RIDER. Optical density was defined at 450 nm wavelength. Relationship between optical density and cytokines' concentrations were defined using the standard curve developed by special computer program TITERSOFT. The following detection kits were used (Bender Med systems Diagnostics GmbH, LOT, 227,228 Renweg 95b, A-1030, Vienna, Austria).

SOD levels were detected by spectrophotometric assay [10].

The data obtained were analyzed by computer software SPSS 10,0. All data were expressed as means \pm SD. Student's t-paired test was used for analysis of differences between means. Normally distributed continuous variables were compared with one-way analysis of variances (ANOVA) and Kruskal-Wallis test was used to compare abnormally distributed variables. Pearson correlation and multiple logistic regression analysis (forward stepwise conditional model) were used, when all researched factors and stroke risk factors entered into the model. Hosmer and Lemeshow test assessed the goodness of fit of each model.

RESULTS

On 14th day after the onset of stroke 10,3% of patients in the 1st group achieved amelioration, 59,7% of patients remained stable and 30% of patients deteriorated. In second group - 65,6% of patients ameliorated, 27,4% of patients were stable and 7% of patients deteriorated. In 1st group – 90,8% of patients ameliorated and 9,2% of patients remained stable (Fig.1). The initial indexes of anti-inflammatory cytokine IL-10 were elevated in all 3 groups compared control, while the 1st group revealed significantly diminished levels of IL-10 against two other groups ($p < 0,05$). On 14th day of stroke onset the levels of anti-inflammatory cytokine IL-10 were significantly elevated in comparison with initial data ($p < 0,05$). Statistical differences were found between means of 1st group and two other groups, while the high standard deviations in the 1st and 2nd groups indicated to the individuals with higher IL-10 levels in the mentioned groups, which found to have the ameliorated neurological state (Fig.1). ANOVA analysis between individuals of the same group (1st) found 3 patients (10,3%) with relatively high levels of IL-10 ($p < 0,05$) with better neurological outcome at 14th day of stroke. ANOVA analysis found statistical differences between 38,5% individuals of 2nd group ($p < 0,05$) with amelioration, though the levels of IL-10 in 26,5% of patients with amelioration were non-significantly higher. Differences regarding the IL-10 levels on 14th day of stroke onset were not found between individuals of 3rd group (Tab.1).

The initial indexes of blood SOD were non-significantly elevated in 1st group compared control and found to be significantly higher in 2nd and 3rd groups ($p < 0,05$). Significant differences were revealed between 2nd and 3rd groups ($p < 0,05$). On 14th day of stroke onset the levels of SOD significantly increased in all 3 groups compared to initial data ($p < 0,05$). Statistical differences found between means of all three groups with prevalence

of 3rd group compared 2nd group, while the 1st group found to have the decreased levels of SOD compared 2nd group ($p < 0,05$). Again, individual cases with higher levels of SOD in 2nd and 3rd groups showed the ameliorated neurological state on 14th day from disease onset. ANOVA analysis held in the same groups, between individuals of 1st and 2nd groups found statistical differences in mentioned 3 patients with ameliorative course in 1st group ($p < 0,05$) and in 47% of patients in 2nd group. Multivariate logistic regression of all above enlisted risk-factors of stroke and researched indexes found significance of IL-10, SOD and for arterial hypertension in combination with high cholesterol levels for neurological outcome at 14th day of stroke onset. Significant negative correlation (Fig.3) was found between IL-10 and SOD levels and NIHSS score at 14th day ($r = -0,28$; $p < 0,05$; and $r = -0,31$; $p < 0,05$ respectively). Significant positive correlation was found between hypertension in combination with high cholesterol levels and NIHSS score at 14th day from stroke onset.

DISCUSSION

It is well established that local inflammation of microglial cells in penumbra tissue plays the key role in fundamental mechanisms of ischemic neuronal injury. The activated microglial cells produce the proinflammatory cytokines: interleukine-1 (IL-1), interleukine-6 (IL-6) and tumor necrosis factor- α (TNF- α), which influence the function of other cytokines by a complex cytokine network and trigger the recruitment of leukocytes into CNS. Activation of proinflammatory cytokines leads to the adhesive and thrombotic events in ischemic microvessels, and thus, microcirculation disorders in penumbra tissue. Transendothelial migration of leukocytes through damaged blood-brain barrier and secondary tissue infiltration results in formation of lipid metabolites, free toxic radicals and reactive oxygen species, which in turn induce the oxidative/peroxidative reactions leading to the irreversible neuronal damage [5]. Free radical pathology along with inflammation and excitotoxicity trigger the pathways of necrosis and apoptosis through various biochemical cascades. Activation of endogenous bio-protective system resists to the impact of brain ischemia and protects the neuronal cells from irreversible injury. Ultimate adverse effects of pro-inflammatory cytokines, excitotoxicity and free oxygen and nitrogen radicals depend on levels of antioxidative enzymes, free radical scavengers, growth factors, glucose, anti-inflammatory cytokines and etc. in the environment of brain cells [3,4]. Experimental models of MCA occlusion (Wistar rats) revealed the different ischemic brain lesion sizes in dependence with time of occlusion and probably with genetically determined protective mechanisms [11]. Usually, production of anti-inflammatory cytokines (IL-4, IL-10) gradually increases after 2-3 days from acute brain ischemia that limits the adverse effects of proinflammatory agents [4]. It can be supposed that in severe ischemia cases and in the cases of immunodeficiency production of anti-inflammatory agents and enzymes-antioxidants is strongly suppressed. Several publications favor such point view indicating that transcriptional regulation of macrophagal NO-synthase (NOS-2) is violated in conditions of immunodeficiency [8,9].

In presented research the differences found between means of IL-10 and SOD of different groups in acute and

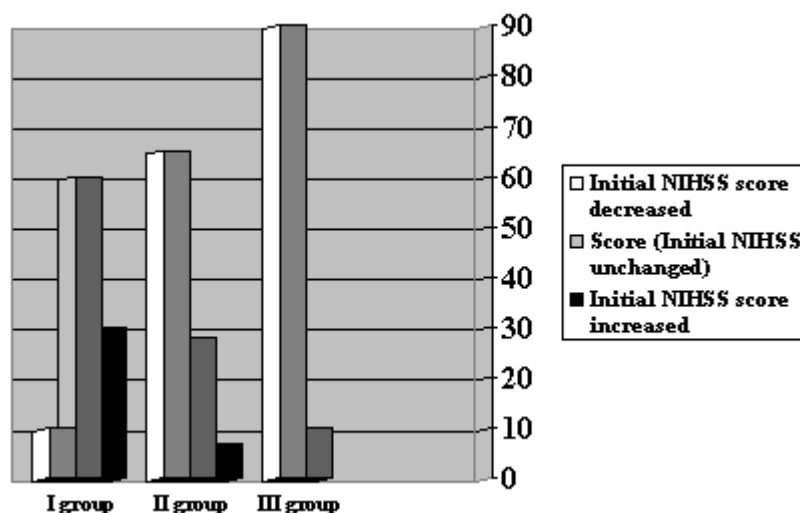
sub-acute stage of stroke can be explained by above mentioned suppositions. Relatively high production of blood IL-10 and SOD with better neurological outcome even in severe stroke cases can be attributed to the high activity of endogenous protective system which is probably genetically determined [14]. Recently, large

population studies revealed the genetic polymorphism of IL-10 that might play a pivotal role in critical situations [7]. Apparently, anti-inflammatory cytokines and enzymes-antioxidants, which now are under experimental trials in future will be used in clinical stroke treatment.

	48 hours				14 th day			
	Control	1 st group	2 nd group	3 rd group	1 st group	2 nd group	3 rd group	3 rd group
% of Patients					89,7%	10,3%	61,5%	38,5%
IL-10 pg/ml	5,6±1,2 3	*6,4±1,2	*7,1±1,2	7,0±1,4	9,4±0,8	*13,8±0,6	14,6±0,3	*15,9±0,2 16,2±0,8
% of Patients					89,5%	10,3%	53%	47%
SOD µmol/l	0,018± 0,001	0,021± 0,003	*0,038± 0,001	*0,051± 0,004	0,046± 0,002	*0,059± 0,001	0,072± 0,002	*0,081± 0,002 *0,082± 0,003
NIHSS		18±3	*12±2,5	*5±3,5	18±3	*15±3,5	12±2,5	*9±2,5 * 3±1,5

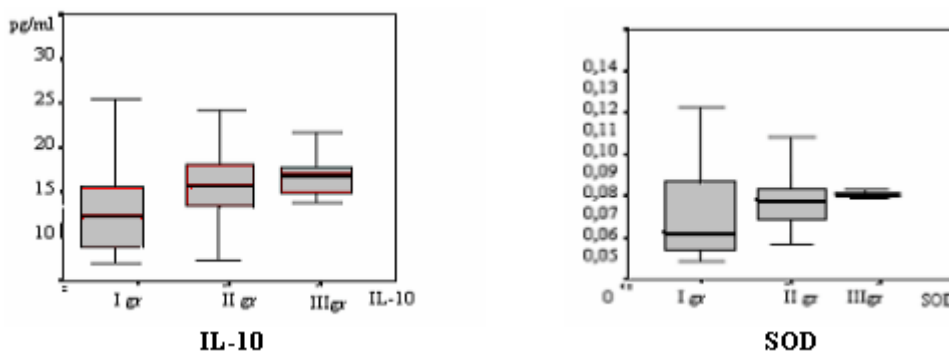
Note: Numbers represent means (SD); * - p<0,05

Tab.1 Differences between blood levels of IL-10 and SOD at initial stage and at 14th day of stroke between groups and in the same groups.



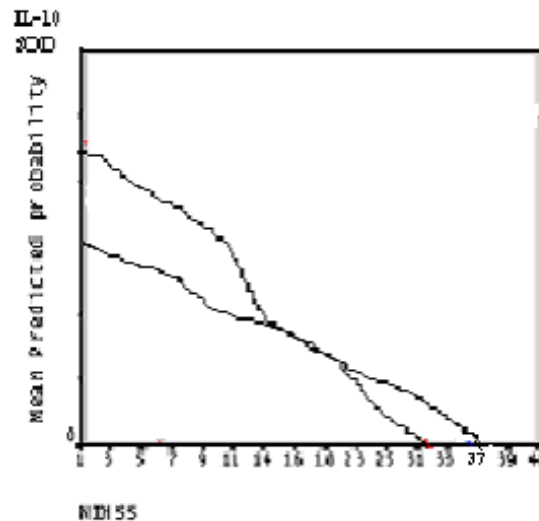
Note: Increasing the NIHSS score from baseline NIHSS is considered as deterioration. Decreasing the NIHSS score from baseline NIHSS is considered as amelioration.

Fig.1 The percent of patients with ameliorative, stable and deteriorated neurological outcomes in different groups at 14th day of stroke onset.



Note: For IL-10: Chi-square -94.999; degree of freedom (df)-3; Sig. -0.000. For SOD: Chi-square -92.730; degree of freedom (df)-3; Sig. -0.000.

Fig.2 The box plots represent the comparisons of means and standard deviations (ANOVA) of IL-10 and SOD levels between groups on 14th day of stroke onset.



Note: The upper line represents tendencies for IL-10; Sig. -0.000; Standard error (S.E.)- 0.059, degree of freedom (df)-1.
The lower line represents tendencies for SOD; Sig.- 0.000, S.E. -0.022, (df)-1.

Fig.3 Multivariate logistic regression model (entered stepwise) represents the significant negative relationship between IL-10 and SOD levels and NIHSS score at 14th day of stroke onset.

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Отношение некоторых индексов эндогенной защитной системы к неврологическому исходу заболевания на 14-й день после развития ишемического инсульта

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РЕЗЮМЕ

Целью данного исследования являлось изучение некоторых показателей эндогенной защитной системы в остром и подостром периоде ишемического инсульта и отношение этих показателей к неврологическому исходу заболевания на 14-й день. Обследовано 95 больных с острым ишемическим инсультом. Для определения тяжести заболевания использовались международные шкалы NIHSS и GCS. Больные были подразделены на 3 группы: 1) с тяжелым инсультом (GCS<9, NIHSS>15), 2) со средней его тяжестью (GCS=14-15; NIHSS=10-15) и 3) со сравнительно легким инсультом (GCS=15, NIHSS<10). Неврологический статус оценивали на 14-й день по NIHSS. Содержание интерлейкина-10 (IL-10) в крови определяли методом иммуноферментного анализа ELISA 48 часов спустя и на 14-й день после развития инсульта. Содержание супероксидсмутазы (СОД) исследовали спектрофотометрически. Статистическую обработку данных проводили при помощи компьютерного пакета SPSS. Применяли мультивариантную логистическую регрессию и корреляционный анализ ANOVA. На 14-й день после развития инсульта содержание IL-10 и СОД в крови повышалось во всех группах в сравнении с изначальными и контрольными величинами ($p<0,05$), тогда как данные в группе больных с тяжелым инсультом достоверно были понижены в отличие от групп больных со средней тяжестью и со сравнительно легким течением инсульта ($p<0,05$). Значительная отрицательная корреляция выявлена между содержанием IL-10 и СОД в крови и неврологическим исходом по NIHSS на 14-й день ($r=-0,28$ $p<0,05$ и $r=-0,31$ $p<0,05$ соответственно). По видимому, неврологический исход инсульта к концу второй недели в значительной мере зависит от состояния эндогенной защитной системы.

Ключевые слова: интерлейкины, воспаление, пенамбра, ишемия, биопротекция

□ International committee of medical journal editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126:36-47.

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