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Clinical and Biochemical Efficiency of Neuroprotective Therapy in Patients with Chronic Cerebral Ischemia

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Abstract

Objective: to evaluate the clinical and biochemical efficiency of Cortexin as to its impact on the activity of antioxidant enzymes, the content of the reduced forms of SS-SH system and cognitive functions in patients with chronic cerebral ischemia (CCI).

Material and methods: There were investigated 60 patients with CCI (41 women and 19 men) at the ages from 40 to 73 years (average age - 56,52 ± 8,09 years). Clinico-neuropsychological, laboratory and instrumental methods of examination were used in the given work. Patients, depending on treatment regimen, were divided into 2 groups: main group (n = 30) and control (n = 30). Groups of patients were compared by age, sex, education, duration of disease. Patients of main group on the background of basic therapy used Cortexin 10.0 mg intramuscular dose for 10 days; patients of control group received treatment under a clinical protocol, but without the use of Cortexin. The patients of main and control group were investigated three times - at the beginning of treatment, 10 days after and after 1 month.

Results. In dynamics of Cortexin treatment of patients with CCI the severity of cognitive impairment was significantly reduced, as evidenced by the positive dynamics of neuropsychological testing results. Clinical efficiency of Cortexin in patients with CCI was accompanied by positive dynamics of biochemical parameters in the glutathione system in plasma and hemolysate of erythrocytes. After 10 days of Cortexin treatment, glutathione reductase (GR) and glutathione peroxidase (GPO) activity in plasma of patients with CCI have been

increased significantly, and activity of GR and content of SH-groups and reduced glutathione (RG) have been decreased. Reliable improvements in activity of glutathione transferase (GT) and GPO have been kept stable during the following month after treatment completion. More steady increases in glutathione system of patients with CCI were observed in hemolysate of red blood cells. Significant changes of investigated indicators were found not only after Cortexin treatment completion (2nd visit), but this positive trend was stable for a month after the end of treatment (3rd visit), excluding GT activity. There was determined steady increase of RG in hemolysate of red blood cells in patients with CCI against lack of its significant changes in plasma during the observation period.

Conclusions. The use of neuroprotective drug Cortexin for patients with CCI significantly improved their cognitive function, which was confirmed by a comprehensive neuropsychological testing. Cortexin course of treatment significantly affected the state of glutathione system, as in the plasma of patients and in hemolysate of erythrocytes.

Keywords: chronic cerebral ischemia, cognitive function, reduced glutathione, glutathione-dependent enzymes, Cortexin

Introduction

Cerebrovascular diseases are one of the most significant problems of neuroscience, due to the high prevalence, disability and mortality rate from this pathology [1, 2, 5, 7]. Cognitive and associative deterioration in terms of cerebral ischemia occurs in the setting of significant structural changes in the brain tissue that were caused by inhibition of biological combustion processes, enlargement of glutamate “exit toxicity”, hyper production of reactive oxygen species, decreased activity of antioxidant systems and arousal of apoptosis [2].

Increased production of reactive oxygen species in the ischemic brain, which occurs at lower antioxidant functional activity of the neuron system, leads to the enlargement of oxidative stress. In this area it is very important to study antioxidant system, in general mechanisms of which the low-molecular and high-molecular thiol-containing compounds take the lead. Thiol-containing compound are molecules that include SH-groups and are well represented in the cell as a tripeptide glutathione and numerous proteins [12]. Glutathione, which has a crucial role in the vital cell and the organism in whole, it is a key intracellular antioxidant [13]. In conditions of ischemia and inhibition of glutathione-dependent enzymes, there are following processes as oxidative modification of low-molecular thiols, homocysteine formation and, consequently, abnormalities of nitric oxide transport and the formation of cytotoxic derivatives, which enhance more thiols oxidation. The presence of very active thiol antioxidant system in neuron, which is able to regulate the transport of nitric oxide, and it provides the cell resistance to nitrosative stress as the earliest neuro-destructive mechanism in ischemia [11]. We know there were changes from the side of thiol antioxidant system,

which were shown in the reduction of regenerated forms and increasing of oxidized forms, these changes are some of the early signs of cerebral ischemia [3].

For today, one of the most promising treatments for chronic cerebral ischemia (CCI) is neuroprotective therapy that focuses on metabolic neuron protection. Practical implementation of new classes of pharmacological agents, that can influence the different links of pathogenesis and optimize cerebral metabolism, will stop the progression of cerebrovascular disease [2]. Therefore, especially important is to use neuroprotective agents that combine the antioxidant, antiischemic and nootropic properties [2, 7, 9].

Finding of peptide neurotrophic factors prompted the researchers to form new strategies in the pharmacotherapy of diseases in the central nervous system - peptidergic or neurotrophic therapy. Products of this group include Cortexin [7]. Cortexin is a complex of amino acids, polypeptides and trace elements released from the calves' cortex and it has important role in neurons activity, formation of neuroprotection mechanisms [1, 6].

Experimental studies have established that the basis of neuroprotective and nootropic actions of Cortexin is its ability to reduce mitochondrial dysfunction and neyro apoptosis - complex pathological processes leading to persistent cognitive impairments [2]. Clinical studies have shown the effectiveness of Cortexin in impacting on cognitive function in patients with cerebral stroke [4], CCI [5], craniocerebral trauma [9]. Improvement of cognitive function is associated with increased functional activity of frontostriatal and thalamocortical relations [10]. In addition, the literature shows the results of Cortexin positive impact on the state of glutathione-dependent link in antioxidant system of red blood cells hemolysate in patients with craniocerebral trauma acuity [9]. That's why it is important to investigate neuroprotective efficiency of Cortexin as to its impact on the state of the glutathione system in patients with CCI.

Objective: to evaluate the clinical and biochemical efficience of Cortexin as to its impact on the activity of antioxidant enzymes, the content of the reduced forms of SS-SH system and cognitive functions in patients with CCI.

Materials and research methods

There were investigated 60 patients with CCI (41 women and 19 men) at the ages from 40 to 73 years (average age - 56,52 ± 8,09 years). Etiological factors of the disease were cerebral vascular atherosclerosis and its combination with arterial hypertension. Diagnosis was formulated according to the classification of brain vascular abnormalities ICD-X (International Classification of Deseases) and confirmed with instrumental and laboratory examination data (CT/MRT of the brain, duplex scanning of brachiocephalic vessels, fundus examination, lipid profile, coagulogram).

A neuropsychological testing of patients with CCI was conducted with help of brief grading scale of higher mental functions - Montreal scale of Cognitive Assessment (MoCA), Frontal Assessment Battery (FAB) tests to evaluate frontal lobe dysfunction, Clock Drawing Test (CDT).

In order to conduct laboratory studies, blood sampling was run in median cubital vein from 8.00 am to 9.00 am on an empty stomach after 12-hour restraint from food. The concentration of reduced glutathione (RG) in plasma and hemolysate of red blood cells of investigated patients of main and control group were determined fluorometrically. The content of SH-groups and activity of glutathione-dependent enzymes (glutathione transferase (GT), glutathione reductase (GR) and glutathione peroxidase (GPO)) in plasma and hemolysate of red blood cells of patients were determined spectrophotometrically [8]. The activity of all investigated enzymes in the plasma was transferred per gram of protein, and in hemolysate of erythrocytes - per gram of hemoglobin (Hb).

Patients, depending on treatment regimen, were divided into 2 groups: main group (n = 30) and control (n = 30). Groups of patients were compared by age, sex, education, duration of disease. Patients of main group on the background of basic therapy used Cortexin 10.0 mg intramuscular dose for 10 days; patients of control group received treatment under a clinical protocol, but without the use of Cortexin. The patients of main and control group were investigated three times - at the beginning of treatment, 10 days after and after 1 month.

In order to assess the effectiveness of Cortexin there was used Clinical Global Impression scale - Clinical Global Impression scale (- 3 - significant deterioration, - 2 - moderate deterioration, - 1 - marginal deterioration, 0 - no change, + 1 – marginal improvement, + 2 - moderate improvement + 3 - a significant improvement) [14].

Statistical methods. Research results were processed under Statistical Package license applications «STATISTICA® forWindows 6.0» (StatSoftInc., USA, № AXXR712D833214FAN5), and «Microsoft Excel 2010". Normality of distribution parameters was established by the criterion Shapiro-Wilkie. These descriptive statistics are represented as the arithmetic mean and standard deviation - M ± SD or median and interquartile range - Me (Q1-Q3) in accordance with distribution characteristics. Pairwise comparison of two related samples was performed under parametric Student's t-test, Friedman nonparametric methods for three linked samples (in the dynamics of treatment) and then there was used the Wilcoxon T-test. Differences were considered significant at $p < 0.05$.

Results and discussion

In dynamics of Cortexin treatment of patients with CCI the severity of cognitive impairment was significantly reduced, as evidenced by the positive dynamics of neuropsychological testing results (Table 1). Thus, there was significant increasing of evaluation overall total scores for scales MoCA, FAB and CDT. And in control group of patients there was significant increasing of overall composite score on a scale only MoCA.

Table 1, Dynamic pattern of neuropsychological testing rates in the treatment of patients with CCI, in points

Scales, tests	Groups of patients	Before treatment (1st visit)	After treatment (3rd visit)	p
MoCA	Main	24,73±2,16	25,80±2,04	<0,0001
	Control	25,00±2,41	25,23±2,45	0,01
FAB	Main	16,00 (15,00-17,00)	17,00 (16,00-18,00)	0,0003
	Control	16,00 (14,00-17,00)	16,50 (14,00-17,00)	>0,05
CDT	Main	7,50 (4,00-9,00)	9,0 (8,00-10,00)	0,0003
	Control	7,00 (4,00-9,00)	7,50 (5,00-9,00)	>0,05

Clinical efficiency of Cortexin in patients with CCI was accompanied by positive dynamics of biochemical parameters in the glutathione system in plasma (Table 2) and hemolysate of erythrocytes (Table 3). Thus, after 10 days of Cortexin treatment, GR and GPO activity in plasma of patients with CCI have been increased significantly, and activity of GR and content of SH-groups and RG have been decreased. Reliable improvements in activity of GT and GPO have been kept stable during the following month after treatment completion.

More steady increases in glutathione system of patients with CCI were observed in hemolysate of red blood cells (see Table 3). Significant changes of investigated indicators were found not only after Cortexin treatment completion (2nd visit), but this positive trend was stable for a month after the end of treatment (3rd visit), excluding GT activity. There was determined steady increase of RG in hemolysate of red blood cells in patients with CCI against lack of its significant changes in plasma during the observation period.

Table 2, Dynamic pattern of glutathione system parameters in plasma during the treatment of patients with CCI

Indexes	1st visit	2nd visit	3rd visit	p	p_{1-2}	p_{1-3}	p_{2-3}
Main group (n=30)							
GT mcmol/ (min*g protein)	1,81 (1,63-2,07)	1,49 (1,26-1,70)	1,56 (1,33-1,77)	<0,0001	0,0003	0,001	>0,05
GR mcmol/ (min*g protein)	0,46 (0,40-0,62)	0,62 (0,52-0,79)	0,55 (0,45-0,69)	>0,05	0,005	>0,05	>0,05
GPO mmol/ (min*g protein)	2,01 (1,81-2,22)	2,31 (2,03-2,49)	2,18 (1,94-2,52)	0,039	0,001	0,049	>0,05

Table 2, (Continued): Dynamic pattern of glutathione system parameters in plasma during the treatment of patients with CCI

SH-groups mcmol/ g protein	19,43 (16,19-21,12)	16,98 (15,11-18,67)	17,18 (16,43-18,96)	>0,05	0,039	>0,05	>0,05
RG mcmol/l	26,05 (21,20-28,50)	24,40 (22,80-25,80)	24,05 (21,80-25,60)	>0,05	0,032	>0,05	>0,05
Control group (n=30)							
GT mcmol/ (min*g protein)	1,94 (1,68-2,19)	1,79 (1,45-2,11)	1,74 (1,61-2,02)	>0,05	>0,05	>0,05	>0,05
GR mcmol/ (min*g protein)	0,48 (0,40-0,65)	0,65 (0,41-0,77)	0,56 (0,44-0,66)	>0,05	>0,05	>0,05	>0,05
GPO mmol/ (min*g protein)	2,03 (1,76-2,28)	2,19 (1,88-2,39)	2,10 (1,93-2,31)	>0,05	>0,05	>0,05	>0,05
SH-groups mcmol/ g protein	19,05 (14,77-22,61)	20,69 (18,73-23,27)	20,17 (17,36-23,20)	>0,05	>0,05	>0,05	>0,05
RG mcmol/l	26,05 (21,50-29,40)	24,60 (21,70-26,70)	23,45 (21,50-27,40)	>0,05	0,023	>0,05	>0,05

Table 3, Dynamic pattern of indicators in erythrocyte glutathione of hemolysate in patients with CCI during the treatment

Indexes	1st visit	2nd visit	3rd visit	p	p_{1-2}	p_{1-3}	p_{2-3}
Main group (n=30)							
GT mcmol/ min* g Hb)	3,79 (2,77-5,27)	3,19 (2,41-4,06)	3,25 (2,04-4,32)	>0,05	0,012	>0,05	>0,05
GR mcmol/ min* g Hb)	1,78 (1,49-2,05)	2,24 (1,92-2,54)	2,05 (1,65-2,23)	0,001	0,0003	0,049	0,01
GPO mmol/ min* g Hb)	17,52 15,45-22,09)	22,66 (17,76-27,65)	20,00 17,41-25,06)	0,024	0,002	0,013	>0,05

Table 3, (Continued): Dynamic pattern of indicators in erythrocyte glutathione of hemolysate in patients with CCI during the treatment

RG mmol/l	2,00 (1,80-2,10)	2,30 (2,00-2,50)	2,15 (1,90-2,30)	<0,0001	0,0003	0,026	0,0001
Control group (n=30)							
GT mcmol/ min* g Hb)	3,38 (2,49-5,34)	3,18 (2,29-4,25)	3,22 (2,26-4,37)	>0,05	>0,05	>0,05	>0,05
GR mcmol/ min* g Hb)	1,77 (1,68-1,96)	1,97 (1,55-2,28)	1,87 (1,61-2,53)	>0,05	>0,05	>0,05	>0,05
GPO mmol/ min* g Hb)	17,11 14,94-20,13)	18,61 (14,35-22,06)	18,35 15,82-22,09)	>0,05	>0,05	>0,05	>0,05
RG mmol/l	1,90 (1,70-2,10)	2,00 (1,90-2,20)	2,00 (1,80-2,20)	0,02	>0,05	>0,05	>0,05

Taking into account, that basic therapy in patients with CCI include antihypertensive and hypolipidemic drugs, disaggregants (for the main indication), the obtained results of investigating justify the advisability of Cortexin treatment for patients with CCI and can prevent polypragmasys in the treatment of chronic cerebrovascular disease.

Evaluation of the clinical efficiency of the Cortexin has been done according to the Clinical Global Impression scale. According to this scale in patients who used Cortexin, there was revealed the following: significant improvement - 53.3%, moderate improvement - 36.7%, marginal improvement - 6.7% and no change - 3.3% of patients. During Cortexin treatment of patients with CCI, no side effects or nothing adverse were detected.

As can be seen from the above, the use of neuroprotective drug Cortexin for patients with CCI significantly improved their cognitive function, which was confirmed by a comprehensive neuropsychological testing. Cortexin course of treatment significantly affected the state of glutathione system, as in the plasma of patients and in hemolysate of erythrocytes. Positive changes in the activity of glutathione-dependent enzymes and reduced glutathione in hemolisate of erythrocytes of studied patients were observed for one month after the treatment, which proves the advisability of Cortexin in comprehensive treatment of this patients category.

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