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CLINICAL AND PATHOGENETIC APPROACHES IN THERAPY OF NEUROINFECTIONS

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New domestic product cytoflavin (CFN, STFF «Polisan») is a integrated drug with a pronounced neuroprotective effect. It contains amber acid, riboxin, nicotinamide, riboflavin mononucleotide sodium. CFL has antioxidant and antihypoxic action, positive effect on energy production in cells, reduces the production of free radicals and stops the activity of antioxidant enzymes. Cytoflavin has anti-ischemic effect, improves cerebral blood flow, restores consciousness, including neuroinfections.

Keywords: neuroinfections, cytoflavin, antioxidant system

Новый отечественный препарат цитофлавин (ЦФН, НТФФ «Полисан») — комплексный препорат с выраженным нейропротективным действием. Содержит янтарую кислоту, рибоксин, никотинамид, рибофлавина мононуклеотид натрия. ЦФЛ обладает антиоксидантным и антигипоксантным действием, оказывает положительное действие на энергообразование в клетке, уменьшает продукцию свободных радикалов и останавливает активность ферментов антиоксидантной защиты. Цитофлавин обладает антиишемическим действием, улучшает мозговой кровоток, восстанавливает сознание, в т.ч. при нейроинфекциях.

Ключевые слова: нейроинфекция, цитофлавин, антиоксидантная система

Introduction

The variety of forms, the acuteness and severity of meningitis, a tendency to epidemic outbreak, the probability of adverse outcome represents a challenge for practitioners in terms of diagnosis and emergency treatment. This once again underlines the importance of studying inflammatory diseases of the meningitis.

The problem of diseases of the nervous system proceeding as meningeal syndrome is late diagnosis of meningitis, high mortality rates, the frequency of neurological complications, expanding range of pathogenic agents causing meningitis and encephalomyelitis. The unbalanced treatment, apparently, causes these phenomena. The necessity of etiotropic therapy is doubtless; however, on the early stages of treatment fate of meningitis patients often depends on emergency pathogenic treatment because it prevents the metabolic processes occurring in brain tissue.

Long-term clinical and laboratory studies have shown that severe forms of viral and bacterial infections are characterized by significant intoxication and hypoxia, microcirculatory disorders with lesions in various organs and systems, decreasing of immunobiological resistance and metabolic activity of neutrophils, sensibilization of peripheral blood leukocytes to bacterial and viral allergens. It mostly deals with the formation of reactive oxygen species (ROS) and lipid peroxidation products (LPO), the imbalance in system of the proteolytic control.

The aforementioned mechanisms may be the cause of complications and a prolonged course of disease, recrudescence of chronic and cocurrent diseases. Ischemic effects on the brain lead to destruction of cell membranes of neurons and glial elements, as well as the capillary vessel by failure of intracellular ion homeostasis and metabolism of macroergic compounds. In this regard, it is expected that therapeutic efforts to reduce energy consumption of neurons, will help them survive the unfavorable period and maintain their structure. Obviously, the main place in the treatment of acute ischemia during reperfusion should occupy the metabolic and neuroprotective therapy.

The antioxidant protection system (AOP), consisting of non-enzymatic and enzymatic systems helps to inactivate the negative impact of ROS on cells and tissues. Non-enzymatic components of AOS are low molecular weight compounds (ascorbic acid, uric acid, tocopherol, etc.) and high molecular weight compounds (proteins of plasma). The main enzyme of specific AOD is superoxide dismutase (SOD). Along with the SOD active components of enzyme systems are ceruloplasmin (CP), glutathione peroxidase containing selenium, catalase, as well as methionine sulfoxide reductase that restores methionine residue in the active center of proteinase inhibitor.

The current standard treatment of inflammatory diseases suggests the use of antioxidants (AO). Natural and synthetic AO prevent the activation of free radical reactions, play an important role in the activation of effector cells mediating the immune response, therefore AO may have immunomodulating properties [1]. It is proved that the main metalloenzymes protect the macroorganism against viral and bacterial infections [2,3]. Recent years biologically active substances with a great variety of pharmacological actions — compounds of succinic acid (SA) [4], have been used in clinical practice due to its antioxidant (AO) and cytoprotective properties. One of the most important derivatives of succinic acid is a new domestic preparation cytoflavin (STFF «Polisan»). Cytoflavin (CFL) is an integrated drug with neuroprotective effect, containing succinic acid (10%), inosine (2%), nicotinamide (1%), riboflavin mononucleotide sodium (0.2%). Cytoflavin has antioxidant and antihypoxic action, exerting a positive effect on energy production in the cell, reducing the production of free radicals and restoring antioxidant enzyme activity (AO). The drug activates redox enzymes of the mitochondrial respiratory chain, resynthesis macroergs, improves glucose and fatty acids utilization. And also, cytoflavin has anti-ischemic

Table 1

The duration of clinical symptoms in patients with neuroinfections gainst the background of cytoflavin

Symptoms	Число больных и сроки исчезновения симптомов (день госпитализации)					
	1-3th days		4-6th days		7th day and later	
	MG	CG	MG	CG	MG	CG
Fever	11 (45%)	6 (30%)	9 (35%)	10 (40%)	5 (20%)	4 (20%)
Symptoms of intoxication	8 (30%)	3 (15%)	12 (50%)	7 (35%)	5 (20%)	10 (50%)
Headache	6 (20%)	2 (10%)	13 (55%)	8 (40%)	6 (25%)	10 (50%)
Nausea, vomiting	19 (75%)	15 (75%)	6 (25%)	4 (20%)	0	1 (5%)
Meningeal symptoms	7 (25%)	3 (15%)	14 (60%)	8 (40%)	4 (15%)	9 (45%)

Legend: MG - main group (CFL), CG - control group.

effect, improves coronary and cerebral blood flow, limiting the zone of necrosis and improves metabolic processes in the central nervous system, restores consciousness, reflex disorders and disorders of sensitivity.

The goal of our study was to investigate the therapeutic efficacy of cytoflavin infusion as antihypoxic, antioxidant preparation improving cerebral blood flow in the integrated treatment of neuroinfections in adult patients.

Materials and research methods

The investigation was open, randomized with a clinical control group (compared with preparation nootropil). The study involved patients with severe and moderate course of neuroinfections (meningitis, meningoencephalitis). Two groups of patients were formed: the main one — 30 patients with neuroinfections receiving against a background of basic therapy infusion CFL; and the control one — 30 patients with neuroinfections receiving against a background of basic therapy parenteral nootropil.

Basic therapy comprised parenteral antibiotics (penicillin or chloramphenicol succinate), infusion therapy (physiological salt solution, 5% glucose solution), vitamins B1, B6 and C, aminophylline, if necessary, diuretics (Lasix, mannitol), and glucocorticoid hormones (prednisone). 5% nootropil solution injected intravenously with 10 ml 2 times a day for 10 days.

Cytoflavin injected intravenously with 10 ml of sterile solution with 5% glucose (200-300 ml) 2 times a day for 10 days in a row.

Results and discussion

Among the main group (treated by cytoflavin) 11 patients with neuroinfections had serous meningitis (SM), 8 — bacterial (purulent) meningitis (PM), tick-borne encephalitis (TBE), meningeal form — 6 patients. According to severity: severe course — 7 (2 — died, including 1 with a meningeal form of TBE, 1 — with PM), moderate — 16 and a light — 2 patients. A similar distribution was also among patients in the control group.

Injections of CFL is well tolerated, allergic reactions were not registered. Against the background of CFL main clinical symptoms (symptoms of intoxication — fatigue, sleep disturbance, loss of appetite) passed more quickly in 25 patients with neuroinfections than in control group; and meningeal signs passed significantly faster after treatment with CFL, CFS had less affected the duration of fever and nausea, vomiting (Table 1). Intoxication persisted 7 days after hospitalization in 20% of patients treated with CFL and in 50% of those in the control group; meningeal symptoms preserved in 15% and 45% accordingly.

Blood tests showed that the prescription of CFL did not affect the content of red blood cells, hemoglobin, erythrocyte sedimentation rate in the main (MG) and control (CG) groups and their amounts were comparable after treatment. The content of leukocytes before treatment was 12.0 × 10 per L in main group and 13,1 × 10 per L in control group; after treatment — 7,2 and 9,5 × 10 per L accordingly. The duration of hospitalization for the main group was 51 bed-days, for the control one — 59 bed-days.

The main interest concerned the dynamics of cerebro-spinal fluid (CSF) in patients with neuroinfections depending on treatment type (Table 2). As we can see, the amount of the cells per 1 mcL in MG was significantly lower than in CG and CSF content improved faster. Generally increased cytosis after 10-12 days of treatment has persisted only in two patients (8%) of MG, while in CG — in 6 ones (30%). Therefore, the prescription of CFL caused more benign clinical course of neuroinfections with faster normalized quantitative and qualitative content of CSF.

Table 2

The dynamics of CSF improvement in patients with neuroinfections taking into account treatment type

Normal CSF parameters	CSF parameters after treatment			
	Serous meningitis		Purulent meningitis	
	MG (CFL)	CG	MG (CFL)	CG
Cytosis (to 5 cells per mcL)	21 c/mcL	47 c/mcL	19 c/mcL	53 c/mcL
Protein (0,16-0,33 g per L)	0,33 g/L	0,4 g/L	0,33 g/L	0,33 g/L

Legend: MG — main group (CFL), CG — control group.

Thus, the authors noted good clinical tolerance and harmlessness, as well as high therapeutic efficacy of

the new domestic preparation cytoflavin in treatment of neuroinfections.

EEG studies with regard to type of therapy have been carried on 17 patients treated with CFL, and 20 patients treated with standard therapy.

In MG (CFL) 2 patients had an extremely severe meningoencephalitis and died (at 4th and 7th day of illness). On the background of a coma in both cases EEG contained gross failure of brain electric activity in the form of widespread synchronized delta activity, demonstrating the dysfunction of bulbar parts of the brain stem, the lack of response to functional load, low amplitude.

17 patients of this group with SM (mean age 25 years) has been admitted to the hospital on 1-5th days of illness. Consciousness was preserved in 15 cases, sopor — 2 (having been admitted in the later stages of illness, died). Lumbar puncture showed high CSF pressure in all patients (CSF flowed out by frequent drops). Neurological status: 8 patients had soft neurological sign expressed in nystagmus, symptoms of oral automatism, Babinski's symptom, increased reflexes and anisoreflexia, one patient had weak lower paraparesis, and one patient had peripheral facial nerve paresis.

The treatment with CFL improved condition on the 5th day of therapy, neurological symptoms disappeared in 5-6 days (with the exception of one patient with persistent symptom of paresis of facial nerve — VII), which correlated with improved functional status of the brain on EEG. Each patient underwent computer 16-channel EEG thriply. EEG analysis let us to identify three types of EEG:

1. The hypersynchronous type of EEG with great number of synchronous pathological forms of slow and sharp activity, generally high amplitude as evidence of dysfunction and irritation of stem structures of medium and lower parts of the brain stem (the most severe cases of meningoencephalitis in 6 patients).

2. Amplitude decreasing, desynchronization, lack of rhythm, widespread often and sharp activity as evidence of irritative processes of CNS (in 4 patients).

3. Disorganized low-amplitude activity, alternating with a bilateral paroxysmal bursts of synchronous, sharp, high-amplitude, polymorphic activity as a manifestation of dysfunction of the mid stem structures, synchronized activity is a sign liquorodynamic disorders (5 patients).

Similar correlations of EEG disorders were observed also in control group of patients.

When comparing the EEG recording in the dynamics distinct positive dynamics of the EEG has been registered during treatment with CFL on the 5th day of therapy; further improvement of the brain functional state — on the 10th day of treatment with CFL as a reduction of slow-wave and paroxysmal manifestation activity and increasing of the rhythm index, restoration of its spatial distribution, adequate reactions to functional loads.

In control group improving of EEG picture was slower — on 10-12th days of basic therapy and significantly less, which correlated with clinical outcome.

One of the indicators of therapy efficiency in patients with neuroinfections was researching of AOP, content of main metalloproteins (MP) in the blood serum and cerebrospinal fluid — transferrin (TF), ceruloplasmin (CP), lactoferrin (LF) and SOD.

It was noticed that before treatment serum levels of TF reduced, and the rest MP significantly increased. After therapy with CFL level of TF increased to normal numbers and the content of LF, CP and SOD remained slightly above normal. Taking into account that these two surveys were taken with the difference of only 10-12 days such levels of MP in the serum is only possible due to significant antihypoxic and antioxidant action of cytoflavin that being a derivative of succinic acid has a positive effect on the system of mononuclear phagocytes (SMF), reducing the production of free radicals and restoring antioxidant enzyme activity.

Patients of the control group on a background of basic therapy (on 10-12th days) had opposite changes of serum MT. Thus, the level of TF decreased on another 6.7%, LF — increased on 11.3%. Level of CP slightly decreased, and SOD of plasma increased on 3.5%.

During studying of MP in CSF before treatment the same pattern that in serum was revealed. Thus, the level of TF reduced, and the rest of the MP increased in comparison with norm. After the treatment with CFL level of TF increased to normal numbers and levels of other MPs have dropped to normal ones. It is important to note that the average concentration of the studied MP in CSF were significantly higher than in serum except for the CP, which level was the same as in the serum, but decreased after treatment. Elevated levels of MP in the CSF may be explained by a higher degree of protection of CNS as the most crucial organ of life support. The data obtained indicate that the major antioxidant serum is CP, in contrast to CSF, where this function belongs to the SOD of plasma.

The dynamics of the MP in CSF after basic treatment was significantly different from that of MP in patients after treatment with CFL. The level of TF increased only on 13.5%, LF and CP — decreased on 3%, and plasma SOD increased on 2.4%. The results indicate a persistent inflammation and oxidative stress at the level of the basic structures of CNS, which correlated with the clinical course of disease.

The mechanism of reduction of TF is the same — the development of acute-phase inflammatory response. Increased levels of IL-1 enhance the release of LF by neutrophils, it is LF that captures iron and leads to giposideremii. It can be assumed that lowering of serum iron, accompanying the development of many inflammatory diseases, reduces the concentration of transferrin. Perhaps, copper-bounding protein is involved in this process, since it has expressed iron-oxidating properties: it oxidizes Fe2 + Fe3 +, which fit into the apotransferrin molecule (2).

Lactoferrin plays an important role in intercellular cooperation of phagocytic cells. LF receptors were found on monocytes, macrophages, neutrophils, activated T-lymphocytes and B-lymphocytes. Absorption of LF by mononuclear phagocytes inhibits their ability to form hydroxyl radical and protects cells from autoperoxidation of membranes. LF is a marker for specific granules of neutrophils, there is a set of receptors of these cells on the inner membrane of them. LF secretion from specific

granules is accompanied by activation of the receptor area of the cell due to fitting of protein-receptors into the outer membrane of neutrophil granulocytes. At the same time failure of the bactericidal function of neutrophils is associated with a decreased activity of LF. Iron deficiency and iron-binding proteins (TF, LF) hampers the immune response and function of lymphocytes.

Thus, analyzed metalloproteins (CP, TF and LF) have not only antioxidant properties but also are important components of nonspecific resistance, providing the body resistance to bacterial and viral infections.

Inclusion of cytoflavin in complex therapy of neuroinfections promoted reliable and more significant increase of TF decrease of LF, CP and SOD in comparison with basic therapy. In other words, cytoflavin (by drop intravenous infusion) has a pronounced corrective effect on the content of metalloproteins, promoted more significant stabilization of the antioxidant capacity of serum and CSF (in comparison with basic therapy), which was accompanied by improvement in clinical status of patients.

The proposed set of laboratory parameters is not only describes the state of the antioxidant defense system of the organism, but also allows to objectively assess the results of ongoing specific antioxidant therapy.

Conclusions

Thus, individual differentiated approach in treatment of neuroinfections is necessary for successful therapy. Different clinical forms require different treatments. We recommend cytoflavin in severe and moderate forms of neuroinfections with significant damage of the nervous system as a drug with antioxidant and antihypoxic action:

— Infusion of cytoflavin is good tolerated by patients with neuroinfections and combined with essential drugs. On the background of cytoflavin there was a significant reduction in the duration of the main clinical syndromes of neuroinfections.

— Cytoflavin promoted to the accelerated normalization (on the 5^{th} day treatment) of EEG changes (in comparison with basic therapy — after $10\text{-}12^{th}$ days), namely: index of alpha rhythm appeared and increased, and the number of abnormal delta and theta forms significantly decreased that correlated with clinical improvement.

— High antioxidant and antihypoxic activity of cytoflavin with the restoration of the antioxidant capacity of serum and CSF is confirmed. The mechanism of cytoflavin action is clarified; it was firstly shown that the major antioxidant of serum in patients with neuroinfections is CP, and cerebrospinal fluid — plasma SOD.

— Therapy with cytoflavin increases immunobiological resistance to viral and bacterial infections. Infusion of cytoflavin is recommended for the treatment of patients with neuroinfections in the acute period, as well as in convalescence.

1. Dubinina E.E. and others // Biochemistry. 1992. Vol.57. Vol.12. P.1892-1901.
2. Drobot I.V. Iron-bounding proteins in Y. pseudotuberculosis and infectious mononucleosis in children. Diss. of cand. St. Petersburg, 1992. 20 p.
3. Isakov V.A., Kovalenko A.L., Turkin V.V., Aspel Y.V. Application of new immunotropic and antioxidant preparations in therapy of influenza and acute respiratory disease. Guide for physicians. St. Petersburg-Velikiy Novgorod. 2000. 74 p.
4. Ivinitsky U.U., Golovko A.I., Sofronov G.A. Succinic acid in the metabolic correction of functional state and the resistance of the organism. St. Petersburg, 1998. 82 p.
5. Lobzin Y.V., Pilipenko V.V., Gromyko J.N. Meningitis and encephalitis. Foliant: St. Petersburg, 2006. Series Actual infection.