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Pharmacological Modulation of Heat Shock Protein 70 (HSP70) - Dependent Mechanisms of Endogenous Neuroprotection in Conditions of Prenatal Chronic Alcoholism by Cerebrocurin and Tiocetam

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ABSTRACT:

Pharmacological modulation of Heat Shock Protein 70 (HSP70) - dependent mechanisms of endogenous neuroprotection in conditions of prenatal chronic alcoholism by Cerebrocurin and Tiocetam

Objective: One of the primary reactions of the genome in response to stress is different genesis induction of heat shock proteins – HSP. The purpose of this study was to investigate the concentration of heat shock protein (HSP70) and hypoxia-inducible factor (HIF-1) in the brain of rats undergoing chronic prenatal alcoholism in different periods of ischemia and define the role of these proteins in the implementation of neuroprotective effect of Cerebrocurin and Tiocetam.

Methods: Experiments were carried out on female rats weighing 150-180 g. All animals were on standard food ration of vivarium, with natural alteration of day and night. Rats were recieved from nursery of «Institute of Pharmacology and Toxicology, Academy of Medical Sciences of Ukraine». All experimental procedures and operative interventions were done in accordance with WMA Statement on Animal Use in Biomedical Research. Rats from the 5th to the 20th day of gestation received ethanol in a dose of 6-8 g/kg/day, control rats – isocalorific sucrose solution. Offspring of alcoholized rats immediately after birth during 25 days were injected intraperitoneally Tiocetam (125 mg/kg), Piracetam (125 mg/kg) and Cerebrocurin (0.06 mg/kg), control rats received saline solution. There were 20 infants in each group. Biochemical studies carried out on brain on 26 days of the experiment, for this purpose the animals were decapitated under anesthesia using Thiopental (30 mg/kg, intraperitoneally). Concentration in the brain tissue and HIF proteins and HSP proteins were determined by Western blot analysis.

Results: Study of concentration in brain tissue HIF proteins and HSP-proteins showed that after undergoing prenatal chronic alcoholism there was an observed decrease in concentration of HSP, so HIF-proteins. Course treatment by Cerebrocurin and Tiocetam resulted in statistically significant increased content of HIF and HSP proteins in the brain in comparison with a group of untreated animals. Neuroprotective activity of Cerebrocurin and Tiocetam was observed in reduction of neurological deficit, as evidenced by the statistically significant decrease in the average score on a scale of C.P. McGrow. Cerebrocurin and Tiocetam directly or indirectly can modulate the expression of early response c-fos genes and thus the "run" software adaptation protein synthesis (including HSP and HIF) in neurons with acute cerebral ischemia.

Conclusion: Implementation of the neuroprotective effect of Cerebrocurin and Tiocetam revealed apparently their ability to increase the concentration in the brain tissues of HSP-protein.

Keywords: prenatal chronic alcoholism, Heat Shock Protein 70, cerebrocurin, tiocetam

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INTRODUCTION

Different types of physiological stress (heat shock, radiation, hemodynamic abnormalities, ischemia, oxidative stress, etc.) cause multiple changes in the cells, including protein structure and function[1,2]. One of the primary reactions of the genome in response to stress is different genesis induction of heat shock proteins (HSP). Increased expression of the genes encoding heat shock proteins are regulated by transcription step. Heat shock proteins are called stress proteins, as increased expression of the corresponding genes is often observed in response to stress[3-10]. Their active participation in the most important processes of functioning cells suggests that these proteins play a key regulatory role in providing repair and degradation, and "failure" in the functioning of the "protein machine" - one of the causes of dysfunction and damage of organs and tissues. Heat shock proteins are named according to their molecular weights[11,12]. For example, the most studied the heat shock proteins HSP60, HSP70 and HSP90 belong to a family of proteins with molecular masses of 60, 70 and 90 kDa, respectively[1,2]. There is a class of proteins (chaperones) whose main function is to restore the correct tertiary structure of damaged proteins and the formation and dissociation of protein complexes. Many chaperones are heat shock proteins, whose expression is initiated in response to the increase in temperature or other cellular stresses[13]. Different types of chaperones involved in transporting substances across membranes, such as mitochondria and endoplasmic reticulum, are involved in correcting the potential harm that results from improper protein folding[3].

In recent data on the role of HSP70 to stabilize the hypoxia-inducible factor (HIF-1a), which in conditions of ischemia are responsible for the expression of erythropoietin gene and another approximately 60 genes whose products are involved in processes such as cell proliferation, apoptosis, angiogenesis, stabilization of protein molecules under conditions of oxidative stress[14-17]. Under hypoxic conditions, at least one of the chaperones (HSP70) is displaced from the complex with HIF-1a protein ARNT, which for 20-30 min hypoxia protects the structure factor of the impact of proteolysis. Thus, it can be assumed that HSP70 is capable of increasing the lifetime factor HIF-1a in conditions before and after hypoxia and thus cells necessary for proper response to oxygen deprivation in acute violation of cerebral circulation[18]. At present, there are almost no studies about pharmacological modulation of HSP70-dependent molecular factors for endogenous neuroprotection in chronic alcohol intoxication. There are no approaches to use of neuroprotective drugs with HSP70-dependent effect in the complex therapy of chronic prenatal alcoholism. Thus, we decided to study neuroprotective effects of Cerebrocurin, Tiocetam and Piracetam in alcoholic encephalopathy.

Piracetam enhances the synthesis of dopamine in the brain, increases the content of norepinephrine. It has a positive effect on the processes of metabolism and blood circulation of the brain, by stimulation of the redox processes, increases glucose utilization, increases the body's energy potential, with the participation of ATP and adenylate cyclase. Piracetam can be used for treatment of cerebral atherosclerosis, vascular parkinsonism, other pathological processes with symptoms of chronic cerebro-vascular insufficiency in violation of memory, attention, speech, dizziness, changes in the cerebral circulation, and diseases of the nervous system with decreased intellectual-mental functions[2,14]. Tiocetam is metabolic neuroprotector based on a fixed combination of piracetam and scavenger of reactive oxygen species – thiotriazoline (morpholinium 3-methyl-1,2,4-triazolyl-5-thioacetate). Tiocetam is used in neurological, geriatric, pediatric, and psychiatric practice. It combines in its action nootropic and antihypoxic piracetam action with antioxidant, anti-ischemic, adaptogenic effect of thiotriazoline[2,14]. Cerebrocurin – neuropeptide of new generation, received from embryos of large horned livestock, contains free amino acids (aspartic acid (446 nmol/mg), threonine (212 nmol/mg), serine (268

nmol/mg), glutamic acid (581 nmol/mg), proline (187 nmol/mg), glycine (298 nmol/mg), alanine (346 nmol/mg), valine (240 nmol/mg), isoleucine (356 nmol/mg), tyrosine (109 nmol/mg), phenylalanine (162 nmol/mg), histidine (116 nmol/mg), lysine (253 nmol/mg), arginine (202 nmol/mg), neuropeptides and low-molecular products of controllable proteolysis (proteins S-100, reelin, nerve growth factors), low-molecular fibers, and peptides of embryos of large horned livestock. Cerebrocurin is used in the treatment of patients with residual effects of cerebrovascular accident, chronic dyscirculatory encephalopathy, psychoorganic syndrome with mental impairment, senile and atherosclerotic dementia, Alzheimer's disease[2,14].

Based on the above, the purpose of this study was to investigate the concentration of heat shock protein (HSP70) and hypoxia-inducible factor (HIF-1) in the brain of rats undergoing chronic prenatal alcoholism and define the role of these proteins in the implementation of neuroprotective effect of Cerebrocurin and Tiocetam.

MATERIALS AND METHODS

Animals

Experiments were carried out on female rats weighing 150-180 g. All animals were on standard food ration of vivarium, with natural alteration of day and night. Rats were recieved from nursery of ®Institute of Pharmacology and Toxicology, Academy of Medical Sciences of Ukraine. All experimental procedures and operative interventions were done in accordance with WMA Statement on Animal Use in Biomedical Research.

Prenatal Chronic Alcoholism

Rats from the 5th to the 20th day of gestation received ethanol in a dose of 6-8 g/kg/day, control rats received isocalorific sucrose solution. Offspring of alcoholized rats immediately after birth during 25 days were injected intraperitoneally Tiocetam (125 mg/kg), Piracetam (125 mg/kg), and Cerebrocurin (0.06 mg/kg), control rats received saline solution. There were 20 infant rats in each group.

Biochemical Analysis

Biochemical assays were carried out on the brain of these animals on the 26th day of the experiement, for this purpose the animals were decapitated under anesthesia using Thiopental (30 mg/kg, intraperitoneally).

Concentration in the brain tissue and HSP-, HIF-proteins was determined by Western blot analysis. Proteins were separated in 10% polyacrylamide gel electrophoresis (PAGE). Transfer of proteins from polyacrylamide gel to a nitrocellulose membrane was performed by electroelution for 45 min. Preincubation Western blots were performed in a solution of TBST with 5% skim milk during 1h. Then western blots were incubated with primary monoclonal antibody (Santa Cruz Biotechnology) against HSP 1:1000 dilution during 1 hour. After washing, blots were incubated with secondary antibodies (Santa Cruz Biotechnology), conjugated with horseradish peroxidase (1:2000 dilution) during 1 hour. Detection of HSP70-, HIF-proteins was performed with use of densitometry in the program Adobe Photoshop[19,20].

Neurologic Impairment

Efficiency of pharmacological correction was estimated by the degree of neurological deficit, which was determined using the C. P. McGrow's Stroke Index Scale[21]. Severity of the condition was defined by the sum of the relevant points: 3 points – mild, 3 to 7 points – average degree, and from 7 points or more - severe degree. The animals were tested daily by exposing the amount of points.

Statistical Analysis

Statistical analyses were performed using a carried out with the help of software for statistic data processing STATISTICA® for Windows 6.0 (StatSoft Inc.). The data were represented by sample mean ±

standard error of the mean value representation. The control of distribution normalcy was done in accordance with Shapiro-Wilk test for normality. Differences between experimental groups were compared with one way ANOVA and posthoc Dunnet test. A p value less than 0.05 was considered statistically significant[22].

RESULTS

Biochemical analysis

Biochemical assays showed that concentration of HSP70 and HIF-1 proteins in the brain of animals with prenatal chronic alcoholism was decreased in comparison with intact group (Table 1).

Administration of neuroprotectors (Piracetam, Tiocetam or Cerebrocurin) led to the increasing of the HSP70 and HIF-1 proteins in the neurons of experimental animals (Table 1, Figure 1 and 2). It should be noted that effects of Cerebrocurin and Tiocetam were more pronounced in comparison with Piracetam.

Table 1: Influence of Cerebrocurin and Tiocetam on concentration HSP70-, HIF-1 proteins (Mean±SEM) in the brain of animals with prenatal chronic alcoholism

Groups of animals	HSP70, conv. u	HIF-1, conv. u
Intact	61.8±2.2	28.0±2.1
Control	41.0±2.4	24.2±2.7
Prenatal alcoholism + Cerebrocurin	110.3±8.1*$	71.1±4.7*$
Prenatal alcoholism + Tiocetam	94.2±2.1*	67.1±4.2*$
Prenatal alcoholism + Piracetam	55.2 ±3.0*	39.7±3.1*

*p<0.05 in comparison with control, $p<0.05 in comparison with the group treated Piracetam

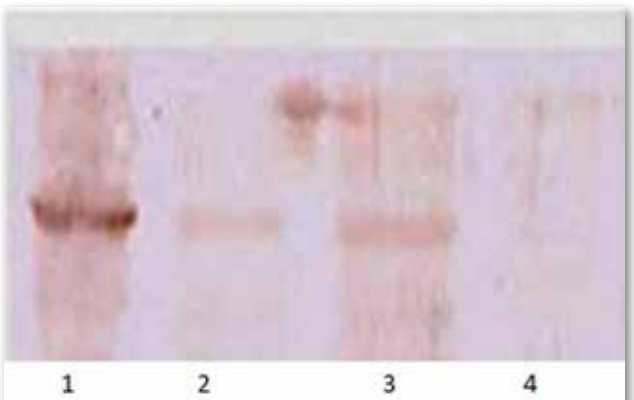

Figure 1: Results of HIF-1 immunodetection (Western blot method): 1– intact animals; 2– group of animals with chronic prenatal alcoholism + Tiocetam; 3– group of animals with chronic prenatal alcoholism + Cerebrocurin; 4– animals with chronic prenatal alcoholism (control group).

Neurologic impairment

In the control group. neurological impairment was defined as an increase of average McGrow scale scores compared to the intact group.

Administration of neuroprotectors led to decreasing of this parameter on 59.50% (Cerebrocurin), on 32.49% (Tiocetam) and on 19.97% (Piracetam) respectively (Table 2).

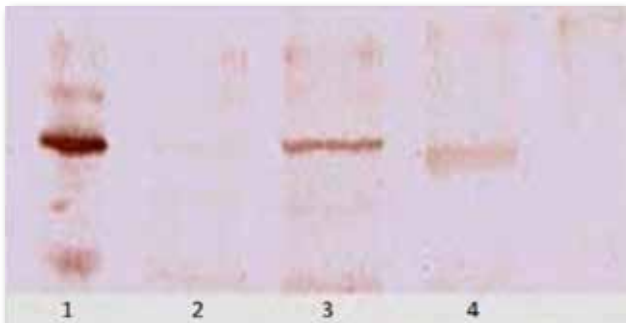

Figure 2: Results of HSP70 immunodetection (Western blot method): 1– intact animals; 2– group of animals with chronic prenatal alcoholism + Tiocetam; 3– group of animals with chronic prenatal alcoholism + Cerebrocurin; 4– animals with chronic prenatal alcoholism (control group).

Table 2: Average scale C.P. McGrow of animals with prenatal alcoholism

Groups of animals (n=10)	Average on a scale McGrow (Mean±SEM)
Intact	0
Control	12.62±1.28
Prenatal alcoholism + Cerebrocurin	5.11±0.52*
Prenatal alcoholism + Tiocetam	8.52±0.63*
Prenatal alcoholism + Piracetam	10.10±1.33*

*p<0.05 in comparison with control

DISCUSSION

Our study of concentration in the brain tissue HIF-, HSP-proteins showed that after undergoing prenatal chronic alcoholism there was an observed decrease in concentration of HSP, so HIF-proteins, which in our opinion, explains the breakdown of

adaptive capacity of the organism in chronic alcohol intoxication, and is due to overproduction reactive oxygen species (ROS), cytotoxic form of nitric oxide, leading not only to the modifications (reversible and irreversible) macromolecules, including the HSP70 and HIF-1 themselves, but also decrease the activity of the expression of genes encoding the synthesis of the latter[17] (Table 1). The protective function of HSP-proteins in CNS pathology (ischemia, hypoxia, neuroinfection, traumatic brain injury) is aimed at both the coordination of linkage of new proteins, correcting of wrong linked, damaged and oxidation modified protein molecules on the transport of proteins across cell membranes, inhibition of protein aggregation, and make degradation by proteasome implementation path.

It is necessary to consider the fact that the HSP-proteins are the major factor of the inductors HIF, which comprises the further adaptive response in the cell. It is shown that HSP- protein is chaperone of the HIF-factor and prolongs its life in conditions of oxygen deficiency. Protein HIF, in turn, forms active dimer with a subunit HIF-1 and starts to play the role of transcription factor, triggering gene transcription as a response to hypoxia. Furthermore, as has been shown by our earlier experimental work, HIF is the induction factor in the synthesis of some antioxidant enzymes[17]. Thus, it can be assumed that HSP70 is capable of increasing the lifetime of HIF factor under hypoxia and thus cells necessary for proper reaction to oxygen deprivation[17,18,23].

Course treatment by Cerebrocurin and Tiocetam resulted in statistically significant increases of content of HIF and HSP proteins in the brain. From Table 1 one can see that the application of Cerebrocurin and Tiocetam increased the content of HSP- and HIF-protein more than 2 times in comparison with a group of untreated animals.

Neuroprotective activity of Cerebrocurin and Tiocetam was observed in reduction of neurological deficit, as evidenced by the statistically significant decreases in average C. P. McGrow scores.

Thus, implementation of the neuroprotective effect of Cerebrocurin and Tiocetam revealed itself apparently with their ability to increase the concentration in the brain tissues of HSP-protein.

In condition of chronic prenatal alcohol brain damage, heat shock proteins (HSP) and factor induced by hypoxia (HIF-1) due to the positive impact on the synthesis of antioxidant enzymes, due to chaperone activity, stabilization of actin filaments, hinder the development of necrosis. In addition, some works have shown the role of increasing the expression of HSP70 in the brain cells (astrocytes) for protecting them from destruction caused by anoxia[1,2,18,23]. It was also demonstrated with the ability of a purified preparation of HSP70 to increase the survival of neurons involved in glutamatergic synaptic transmission in the olfactory cortex of rats from the damaging effects of severe anoxia[12].

Taking into account the HSP's enhancing role in the viability of neuronal cells under hypoxic conditions and the fact that the interaction of HSP and HIF play a pivotal role in the cellular response to hypoxia, we can assume that HSPs are involved in the regulation of signaling pathways of the cell response to hypoxic stress at the level of regulation of stability HIF. It is known by the examined drugs – Cerebrocurin and Tiocetam directly or indirectly can modulate the expression of early response of c-fos genes and hence "run" software adaptation protein synthesis (including HSP and HIF) in neurons with acute cerebral ischemia.

These studies have shown that using Cerebrocurin and Tiocetam on our proposed scheme has a positive impact on the expression of HSP and HIF and significantly increases their effect on concentration in the brain under conditions of prenatal alcoholism. Therefore, Cerebrocurin and Tiocetam showed pronounced neuroprotective effects and these drugs can be recommended for clinical studies in the treatment of prenatal alcoholism.

References:

1. Santoro MG. Heat shock factors and the control of the stress response. Biochem Pharmacol 2000;59(1):55-63. [CrossRef]

2. Belenichev IF, Cherny VI, Buhtiayrova NV, Nagornaya EA, Pavlov SV. Neuroprotection and neuroplasticity. 1st ed. Kyiv: Logos Publishers, 2015. p. 228-316. (Russian)

3. Bar-Or D. Proposed mechanism for induction of heat shock protein 70 by geranylgeranylacetone by prenylation of Ras proteins. Crit Care Med 2003;31(11):2700-1. [CrossRef]

4. Fujiki M, Kobayashi H, Abe T, Ishii K. Astroglial activation accompanies heat shock protein upregulation in rat brain following single oral dose of geranylgeranylacetone. Brain Res 2003;991(1-2):254-7. [CrossRef]

5. Uchida S, Fujiki M, Nagai Y, Abe T, Kobayashi H. Geranylgeranylacetone, a noninvasive heat shock protein inducer, induces protein kinase C and leads to neuroprotection against cerebral infarction in rats. Neurosci Lett 2006;396(3):220-4. [CrossRef]

6. Belenichev IF, Kolesnik Yu M, Pavlov SV, Sokolik EP, Bukhtiyarova NV. Disturbance of HSP70 chaperone activity is a possible mechanism of mitochodrial dysfunction. Neurochemical Journal 2011;5(4):251-6. [CrossRef]

7. Yenari MA, Kauppinen TM, Swanson RA. Microglial activation in stroke: therapeutic targets. Neurotherapeutics 2010;7(4):378-91. [CrossRef]

8. Belenichev IF, Gorbacheva SV, Demchenko NV, Bukhtiyarova NV. The thiol–disulfide balance and the nitric oxide system in the brain tissue of rats subjected to experimental acute impairment of cerebral blood flow: the therapeutic effects of nootropic drugs. Neurochemical Journal 2014:8(1):24-7. [CrossRef]

9. Buhtiayrova N, Odnokoz E. Some aspects of the neurorpotective action of the exogenous HSP70 XXVIth International Symposium on Cerebral Blood Flow, Metabolism and Function & XIth International Conference on Quantification of Brain Function with PET, Abstract Book, 20-23 May 2013, Shanghai, China, p. 584.

10. Fernandez-Funez P, Rincon-Limas DE. Launching Hsp70 neuroprotection: two drugs better than one. Cell Cycle 2014;13(11):1657-8. [CrossRef]

11. Kalmar B, Greensmith L. Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 2009;61(4):310-8. [CrossRef]

12. Doeppner TR, Kaltwasser B, Fengyan J, Hermann DM, Bähr M. TAT-Hsp70 induces neuroprotection against stroke via anti-inflammatory actions providing appropriate cellular microenvironment for transplantation of neural precursor cells. J Cereb Blood Flow Metab 2013;33(11):1778-88. [CrossRef]

13. Shevtsov MA, Nikolaev BP, Yakovleva LY, Dobrodumov AV, Dayneko AS, Shmonin AA, et al. Neurotherapeutic activity of the recombinant heat shock protein Hsp70 in a model of focal cerebral ischemia in rats. Drug Des Devel Ther 2014;8:639-50. [CrossRef]

14. Fedorovich BI, Petrovna SE, Nikolaevich EA. Reduction of apoptotic death of neurons CA-1 zone of hippocampus of rats in the condition of prenatal chronic alcoholisation by cerebrocurin and tiocetam. Elixir Pharmacy 2013;65A:20005-8.

15. Dhar-Mascareno M, Carcamo JM, Golde DW. Hypoxia–reoxygenation-induced mitochondrial damage and apoptosis in human endothelial cells are inhibited by vitamin C. Free Radic Biol Med 2005;38(10):1311-22. [CrossRef]

16. Paul S, Mahanta S. Association of heat-shock proteins in various neurodegenerative disorders: is it a master key to open the therapeutic door?. Mol Cell Biochem 2014;386(1-2):45-61. [CrossRef]

17. Chekman IS, Belenichev IF, Buhtiayrova NV, Gorchakova NA. Atnioxidants: clinical and pharmacological aspects. Ukr med chasopys 2014;3:1-7.

18. Pavlov SV, Belenichev IF. Molecular and biochemical aspects of the neuroprotective effect of the selective estrogen receptor modulator tamoxifen in a model of acute cerebral ischemia. Neurochemical Journal 2014;8(1):28-32. [CrossRef]

19. Adib-Conquy M, Avrameas S, Ternynck T. Monoclonal IgG and IgM autoantibodies obtained after polyclonal activation, show reactivities similar to those of polyclonal natural autoantibodies. Mol Immunol 1993;30(2):119-27. [CrossRef]

20. Beere HM. 'The stress of dying': the role of heat shock proteins in the regulation of apoptosis. J Cell Sci 2004;117(Pt 13):2641-51. [CrossRef]

21. McGraw CP. Experimental cerebral infarction effects of pentobarbital in Mongolian gerbils. Arch Neurol 1977;34(6):334-6. [CrossRef]

22. Zaycev VM, Liflyandskiy VG, Marinkin VI. Applied Medical Statistics: Textbook. 1st ed. Saint Petersburg: Foliant Publishers;2006. p. 169-245. (Russian)

23. Belenichev IF, Cherny VI, Kolesnik YM, Pavlov SV. Rational neuroprotection. 1st ed. Donetsk: Zaslavsky Publishers;2009. p. 32-49. (Russian)