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POSITIVE PHARMACOLOGICAL MODULATION OF HSP70 IN RECOVERY OF BRAIN ENERGY METABOLISM IN VARIOUS MODELS OF CEREBRAL ISCHEMIA

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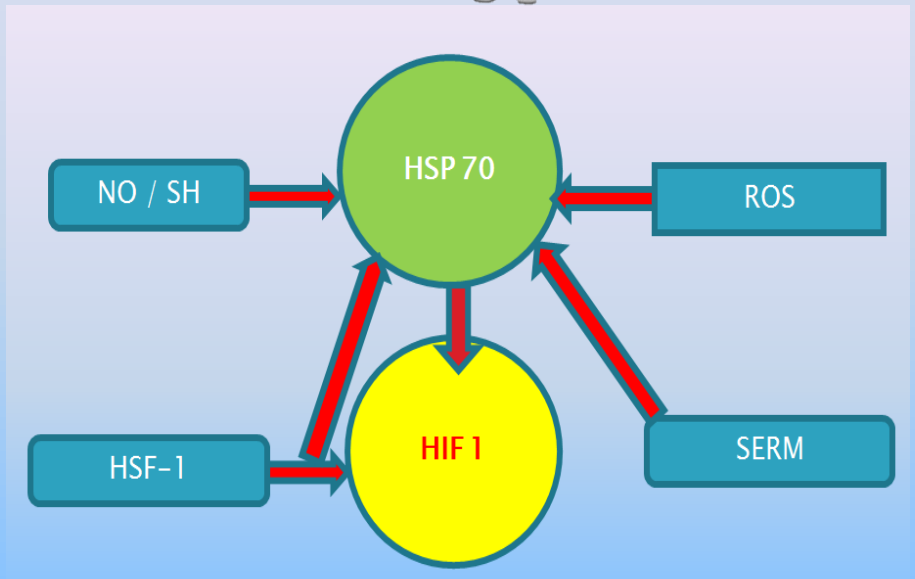
Introduction

Heat shock proteins (HSPs) are an evolutionarily integral part of the functioning of all cells acting as intracellular chaperones that support cell proteostasis under normal and various stress conditions (hyperthermia, hypoxia, oxidative stress, radiation, etc.). HSP70 slows down the mitochondrial and cytoplasmic pathways of apoptosis, inhibits the production of pro-inflammatory cytokines. HSP70 is able to increase the lifetime of the HIF-1a factor under conditions before and after hypoxia and is necessary for cells to properly respond to oxygen deficiency. Under these conditions, they perform a protective function, which is realized through increased synthesis of antioxidant enzymes, stabilization of oxidatively damaged macromolecules, direct anti-apoptotic and mitoprotective action.

Such a role of these proteins in cellular reactions during ischemia raises the question of the development of new neuroprotective agents which are able to provide modulation/protection of the genes encoding the synthesis of HSP 70 and HIF-1a proteins.

Statement of Purpose



The aim of this research was to analyze the neuroprotective action of drugs with an evidence-based effect on the expression of endogenous neuroprotection factors in various experimental models of cerebral ischemia (intracerebral hemorrhage, carotid artery occlusion, prenatal hypoxia) to further substantiate their use in the treatment of CNS damage.

Materials and Methods

The experimental studies were carried out in accordance with the “Regulations on the Use of Animals in Biomedical Research” and with the European Convention on the Protection of Animals Used for Scientific and Other Purposes. The experiment was approved by the Bioethics Committee of Zaporizhzhia State Medical University.

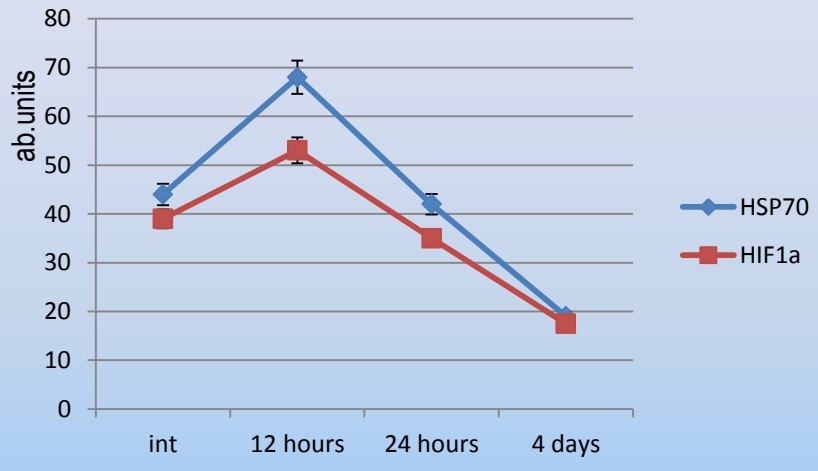
To create an acute cerebrovascular accident (ACVA), a classic model consisting of simultaneous ligation of common carotid arteries was used. The operation was performed with kethaminal-sodium anesthesia (40 mg/kg). Through the incision on neck, the right and left carotid arteries were found and segregated, placed ligatures under them and ligated.

Modelling hematic hypoxia was performed in the prenatal period of development by daily intraperitoneal administration of sodium nitrite solution to pregnant female rats from day 16 to day 21 of the pregnancy at 50 mg/kg. Control pregnant rats received physiological solution in the same regime.

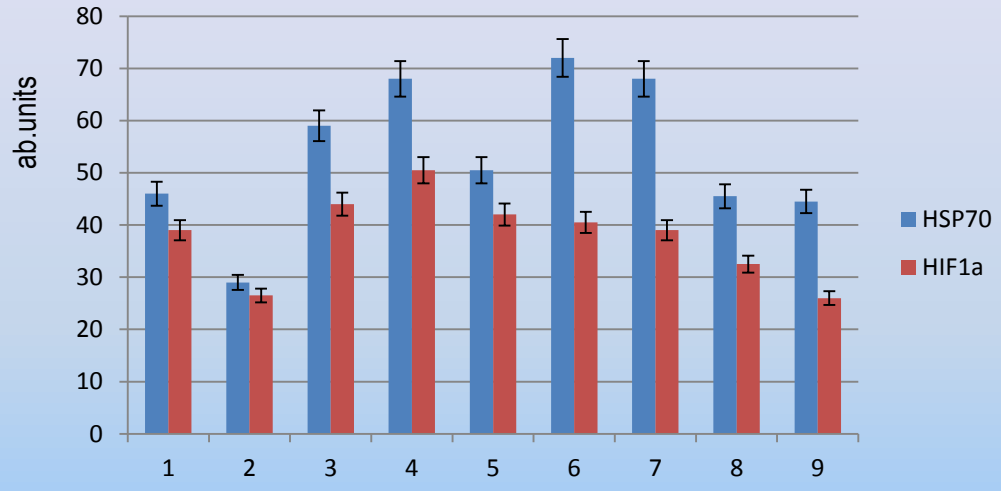
Expression levels of mRNA HSP_{70}, HIF-1, c-fos and the content of HSP_{70} in the cytoplasmic and mitochondrial fractions of the brain of rat on the 60th day of life after PH were determined by real-time PCR and enzyme immunoassay.

Results

The content of HSP70 and HIF-1a proteins in the brain tissues of rats at different times of ACVA



The content of HSP70 and HIF-1a proteins in brain tissues on the 4th day of ACVA after the use of drugs



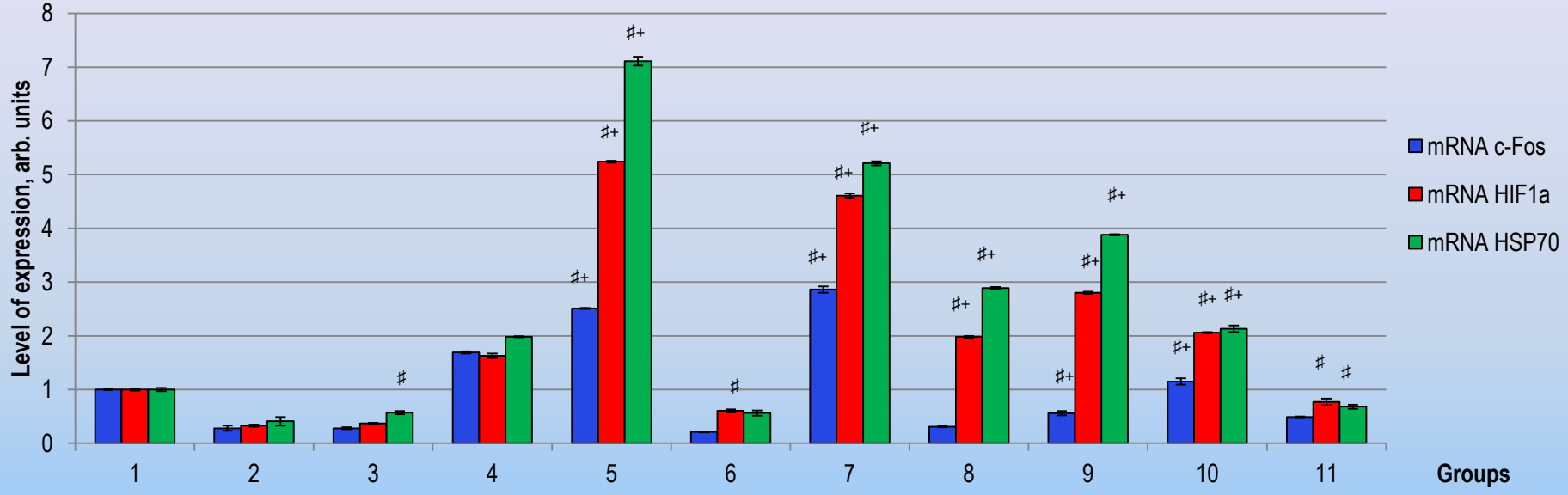
1 - intact, 2 – control, 3 - Thiotriazoline, 4 – (S)-2,6–diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate, 5 - Mexidol, 6 - Tamoxifen, 7 - L-arginine, 8 - Cerebrocurin, 9 - Piracetam

 # - statistically significant differences compared to the control group (PH), $p < 0.05$;

+ - statistically significant differences compared to the Piracetam control group, $p < 0.05$.

Results

Expression of c-fos mRNA, HIF1α mRNA and HSP70 mRNA in the sensorimotor cortex of rats after PH



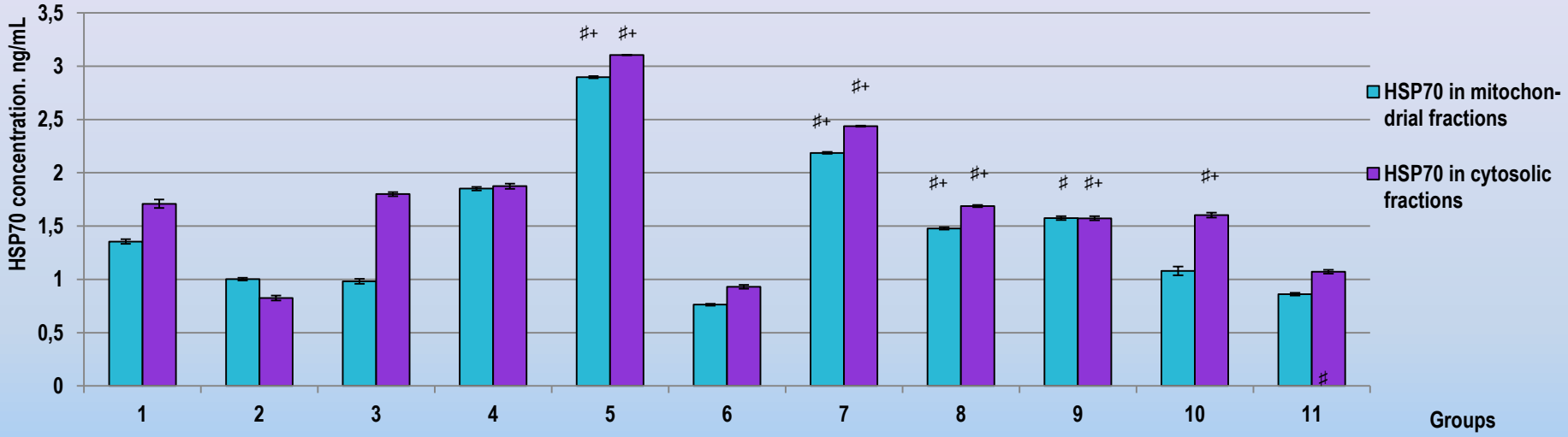
1 - intact, 2 – PH (control), 3 - PH + L-arginine (200 mg/kg), 4 – PH + Tamoxifen (0.1 mg/kg), 5 - PH + Cerebrocurin (150 µl/kg), 6 - PH +Piracetam (500 mg/kg), 7 - PH + (S)-2,6–diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (50 mg/kg), 8 - PH + RAIL (selective IL-1b antagonist) (1 mg/kg), 9 - PH + Glutoredoxin (200 µg/kg), 10 – PH +Thiotriazoline ((50 mg/kg), 11 – PH + Mexidol (100 mg/kg).

♯ - statistically significant differences compared to the control group (PH), $p < 0.05$;

+ - statistically significant differences compared to the Piracetam control group, $p < 0.05$.

Results

HSP70 Concentration in Mitochondrial and Cytosolic Fractions of Rat Brain Homogenate



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♯ - statistically significant differences compared to the control group (PH), $p < 0.05$;

+ - statistically significant differences compared to the Piracetam control group, $p < 0.05$.

Results

- Pharmacotherapy of emerging mitochondrial disorders with modulators of HSP70 expression - Cerebrocurin (composition: neuropeptides, S-100 proteins, reelin, factor nerve growth (NGF)), Glutoredoxin, Tamoxifen, (S)-2,6-diaminohexanoic acid, 3-methyl-1,2,4-triazolyl-5-thioacetate significantly ($p<0.05$) stimulates energy production brain.
- The most pronounced energotropic effect was shown by cerebrocurin (150 µl/kg) and (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (50 mg/kg).
- On the 21st day of treatment of rats with cerebral ischemia, there is a gradual disappearance of severity between animals with simulated pathology and healthy rats, which is associated with actively recovering mitochondrial energy production and cerebral load in general.
- These changes in energy metabolism correlate with the normalization of oxidant-antioxidant balance and the expression of HSP70 mRNA, HIF-1α mRNA and the concentration of HSP70 and HSF-1a in the cytosol and mitochondria of the cerebral ischemia zone and are manifested in a significant ($p<0.05$) decrease in ultrastructural disorders of mitochondria.

Conclusions

- The studied drugs are able to modulate HSP70/Hif1a-mediated mechanisms of endogenous neuroprotection. The most active among HSP70 modulators in conditions of chronic PH are Cerebrocurin (150 µl/kg) and derivative of the diaminohexanoic acid (50 mg/kg), which outperform other studied drugs in terms of increased expression of HSP70 mRNA, HIF-1α mRNA and HSP70 protein concentration in the brain of experimental animals and can be considered as perspective neuroprotective agents in complex therapy after prenatal hypoxia.
- The energy-tropic and mitoprotective effects of the studied drugs are associated with their ability to protect mitochondrial proteins from oxidative damage by increasing the concentration of HSP70, prolong the lifespan of HSF-1a, and activate alternative energy generation pathways.

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Thank you for your attention!

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