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INTRAUTERINE HYPOXIA OF FETUS - INFLUENCE OF ULTRA-LOW DOSES OF ANTIOXIDANT (EXPERIMENTAL RESEARCH)

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Oxygen-sensing mechanisms have been developed to maintain cell and tissue homeostasis, as well as to adapt to the chronic low-oxygen condition, but intensive production of reactive oxygen species (ROS) can cause cell destruction. Previous studies revealed that the hypoxia induces oxidative stress and neurodegeneration, which is associated with memory, behavioral, and learning-education impairment in children. In the view of the above-stated concept, the study of influence of ultra low doses of antioxidant on ROS generation and activity of enzymes of antioxidant protection in a brain and blood at intrauterine hypoxia of a fetus appears appealing.

The effect of Fenozan in ultra low doses was evaluated in the rats underwent intrauterine hypoxia. Research was made on white rats, 66 pregnant females and 279 infant rats (0-21 days). It was established, that chronic pre-natal hypoxia is accompanied by accumulation of malondialdehyde in brain tissue, blood and subcellular fractions of a liver, with the subsequent spontaneous normalization of its maintenance by 21st day in a brain and blood. Fenozan injection in ultra low doses leads to appreciable decrease in MDA level and increase of the ROS-scavenging enzymes at first in a brain and peripheral blood, and then in microsomal and mitochondrial fractions of the liver, that is the precondition for normalization of pathological process in earlier terms. Significance of this data argues that ultra low doses of Fenozan can be less invasive and effective in the treatment of chronic intrauterine hypoxia and suggest the directions for further research.

Keywords: Hypoxia, reactive oxygen species, antioxidant, Fenozan.

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Introduction

Detection of ability of biologically active substances (BAS) to modify live systems of various degree of complexity, operating in ultralow concentration - nanodoses (10^{-22}-10^{-14}M), is one of the most impressing investments of last decades (Burlakova et al., 2008; Alekseeva et al., 2008). There are the facts proving that limitation of the sizes leads to change of conditions for phase and structural transformations, magnetization and demagnetizing, the phenomena of transfer of heat, charge; transmission and reflection of light spectrum and etc. Thus all fundamental characteristics of substance are changed: lattice parameters, an electronic spectrum, energy of electron detachment from an external energetic membrane, temperature of fusion and etc. (Alekseeva, 2008). Forces of an attraction and aspiration to lower free energy create preconditions for self-organizing and self-assemblage of nano-objects and structures, and the nature widely uses with it, especially in bio-objects. The recent researches show that at ultralow concentration the specific molecules of substance can form supramolecular nanodimensional particles, which can be considered as hydrophilic surfaces, and a target of their action in biological objects can be as membrane structures. Natural and synthetic antioxidants concern that type of agents which possess this ability (Burlakova, 2010). Spatially screened phenol Fenozan is the intense antioxidant influencing on structure and functions of membranes. Biologically significant targets of Fenozan influence are microdomains of liposomes,

fragments of a cellular membrane, superficial cellular receptors and endocellular organelle, also system of signal transduction, reparation and apoptosis of cells (Alekseeva, 2010).

The specificities of influence of ultra low doses (ULD) of BAS is complex polymodal dose-effect dependence; kinetic paradoxes in operation of ULD; dependence of reaction on initial characteristics of biological object; «stratification» of effect; increase in sensitivity of bio-objects after ULD action to influence of other physical and chemical factors (Andrievsky, 2010). Mechanisms of these phenomena are actively investigated, however have still not studied up to the end. Considering indispensable involving of damage of membranous brain structures in mechanisms of cellular destruction at hypoxia, it seems tempting that apoptosis, necrosis, necro-apoptosis reveal prospects of antioxidants in ULD for correction hypoxic defeats consequences. In the view of the above-stated concept the study of influence of subminiature doses of antioxidant on level of generation of active forms of oxygen and activity of enzymes of antioxidant protection in a brain and blood, and also in subcellular fractions of a liver has interest at intrauterine hypoxia of a fetus, therefore it became a purpose of this research.

Materials and methods

Research has been provided on white not-purebred rats, 66 pregnant females and 279 infant rats (0-21 days of a life) participated in experiment. The rats-females in chronic experiment had the general hypobaric hypoxia. The animals were isolated in special chamber for 10 times, where within 1 hour 41.1 KPa pressure was created that corresponds to lifting on height 7000м. After parturition the research of newborn infant rats has been organized for 1, 3, 5, 8, 10, 12, 21st days of a life. A slaughter of animals was made by decapitation under ether narcosis. The homogenates were produced from a brain, liver. The secretion medium was 0,125M KCl. The mitochondrial (MC) and microsomal (MS) fractions are produced from liver homogenates with a method of differential centrifugation on Shnaider. Quantity of malon dialdehyde (MDA) was tested by Stalnaya 91977) method, also expressed in nmol/mg of protein x 30 minutes Activity of catalase was tested by permanganatometry on Zubkova (1976). The activity of superoxide dismutase (SOD) was investigated by method of Mirsa and Fridovich (1972) in O.S.Brusov's modification. The synthetic antioxidant Fenozan that synthesised at Institute of chemical physics in Academy of Sciences in Russia, was injected in ULD - (10^{-14} mol/kg) to newborn infant rats of comparison group in 1st, 2nd, and 3rd days of a life in triple way per os. The infant rats of similar age who were not receiving corrections, have made the experiment group, the control group included the animals identical on age, born from healthy females.

Results and discussion

We have revealed, that the increase in level of MDA in brain tissue, blood, mitochondrial and microsomal fractions of liver is observed at the infant rats subjected chronic intrauterine hypoxia. Intensity of increase in this indicator and its dynamics at reoxygenation are not similar in the specified tissues. In homogenate of brain a level of MDA at a birth in the experimental group exceeds the control in 1.4 times (Student's t-test $p<0.001$), progressively increasing to the end of the first days, and then, after a plateau within 3-5 days of a life, gradually decreases to 21st day of supervision, authentically not differing from the control group. It should be noticed, that after a birth in process of development of a brain in rats there is occurred an increase of MDA level which by 21st day is not deferred from a similar indicator at adult rats. The transferred chronic intrauterine hypoxia promotes MDA accumulation in a brain tissue already at the birth moment, and spontaneous fall of MDA concentration is observed only on the 21st day of a life - i.e. to the term corresponding to transition of rats on definitive food. At injection of nanodoses of water-soluble antioxidant Fenozan a decrease in MDA level to a control level is observed earlier - by 10 days. It specifies that antioxidant injection prevents

accumulation of toxic MDA in a brain, providing conditions for normal development of a brain.

TABLE 1. MDA LEVEL (NMOL/MG OF PROTEIN X MINUTES) IN TISSUES OF NEONATAL RATS SUBJECTED PRE-NATAL HYPOXIA, INFLUENCE OF ULD OF ANTIOXIDANT

Brain homogenate								
Group of animals	At birth	24 hours	3rd day	5th day	8th day	10th day	12th day	21st day
Control (n=8-9)	3.86±0.09	4.10±0.11	4.21±0.12	4.15±0.12	4.53±0.11	5.42±0.10	5.43±0.08	5.50±0.09
Experiment (n=8-9)	5.33±0.09	8.34±0.09	7.67±0.07	7.83±0.08	6.12±0.06	5.80±0.12	5.70±0.07	5.57±0.07
Fenozan nanodoses (n=12-16)	abs	8.00±0.12	6.17±0.08	6.19±0.07	5.73±0.08	5.51±0.06	5.49±0.05	5.54±0.05
P1:2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.05	>0.05
P 1:3	-	<0.001	<0.001	<0.001	<0.001	>0.05	>0.05	>0.05
P 2:3	-	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	>0.05
Peripheral blood								
Control (n=8-9)	0.51±0.02	0.55±0.01	0.57±0.01	0.62±0.03	0.60±0.02	0.61±0.02	0.65±0.03	0.77±0.03
Experiment (n=8-9)	1.20±0.05	1.83±0.02	4.62±0.09	3.85±0.04	2.64±0.05	2.43±0.07	1.33±0.04	0.77±0.02
Fenozan nanodoses (n=12-16)	-	1.92±0.06	1.93±0.05	1.00±0.04	0.94±0.05	0.81±0.01	0.70±0.01	0.71±0.02
P1:2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05
P 1:3	-	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	>0.05
P 2:3	-	>0.05	<0.01	<0.01	<0.05	<0.001	<0.001	>0.05
Mitochondrial fraction of liver								
Control (n=8-9)	4.33±0.13	4.77±0.19	5.13±0.10	5.81±0.03	5.60±0.09	5.81±0.06	5.60±0.07	5.72±0.06
Experiment (n=8-9)	5.66±0.07	6.49±0.08	7.74±0.08	7.05±0.04	6.83±0.05	6.96±0.12	6.04±0.06	6.52±0.09
Fenozan nanodoses (n=12-16)	-	6.16±0.06	6.09±0.08	6.08±0.06	5.87±0.10	5.83±0.09	5.78±0.11	5.82±0.15
P1:2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P 1:3	-	<0.001	<0.001	<0.001	>0.05	>0.05	>0.05	>0.05
P 2:3	-	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001
Microsomal fraction of liver								
Control (n=8-9)	0.46±0.04	0.48±0.02	0.68±0.01	0.84±0.01	0.92±0.02	0.87±0.01	0.81±0.02	0.85±0.01
Experiment (n=8-9)	0.85±0.02	3.13±0.06	2.58±0.08	2.66±0.05	2.84±0.05	1.63±0.03	1.41±0.04	1.18±0.05
Fenozan nanodoses (n=12-16)	-	2.08±0.09	1.53±0.04	1.51±0.04	1.00±0.07	0.91±0.03	0.84±0.01	0.87±0.03
P1:2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P 1:3	-	<0.001	<0.001	<0.001	>0.05	>0.05	>0.05	>0.05
P 2:3	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Chronic pre-natal hypoxia is accompanied with MDA accumulation in the peripheral blood, having peak not for 1st days of a life, as in a brain, but for 3rd day of the neonatal period that testifies to active elution of MDA from a brain in blood. Spontaneous normalization of MDA concentration in blood, as well as in a brain tissue, occurs for 21st day. At injection of Fenozan fall of MDA concentration in blood is observed from 3rd day of a life and the effect of Fenozan in blood is more expressed, than in a brain. According to the Table 1, MDA indicators in peripheral blood authentically differ in the experimental group and the group receiving Fenozan in all terms of supervision, except for 1st and 21st days of experiment. It is caused by absence of instant action of Fenozan directly after injection for 1st day and independent restoration of MDA level at the

expense of compensative-adaptive forces of an organism by the 21st day. It should be marked, that at rats of the experimental group and in the group receiving Fenozan , MDA level differs in 2.4; 3.9; 2.8; 3.0 and 1.9 times for 3rd, 5th, 8th,10th, 12th days accordingly (t-test data it is given in the Table 1).

In a liver, organ which is carrying out the central role in detoxication of lipoperoxidation products (Gueraud et al., 2010), MDA level after transferred chronic pre-natal hypoxia is also increased comparing to the control both in MC, and in MS fractions.

In MC fractions of a liver at rats of the experimental group growth of MDA concentration, since the moment of a birth to 5 days of a life, with a maximum on the 3rd day is marked; then its level gradually goes down, remaining authentically high concerning the control for 21st day. At Fenozan injection normalization of MDA level is observed for 8 days, i.e. much faster. Intensity of MDA increase in MC fractions is not too great and makes 1.3-1.5 times.

In MS fractions of a liver at rats of the experiment group the sharp increase in MDA level concerning the control - in 1.9 is observed; 6.5; 3.8; 3.2; 3.1; 1.9; 1.8; 1.4 times according to terms at a birth, for 1st, 3rd, 5th, 8th, 10th, 12th and 21st days (t-test data it is given in the Table 1). Apparently, the peak of increase of MDA corresponds to the 1th day of a life, and in dynamics to the 3rd day its concentration goes down and again increases for 5-8 days, forming an original plateau. Indicator of normalization is occurred on the 21st day. Comparing dynamics of changes of MDA level in MC and MS fractions of liver, it is possible to notice, that a growth of MDA is expressed poorer in MC fraction, than in MS and it is marked later, than in MS fraction where the increase in MDA level is expressed sharply and already on the 1st day after a birth. On the 21st day the spontaneous normalization of MDA is not observed either in MC, or in MS, whereas in a brain tissue and blood in this term MDA concentration is comparable with control sizes. It specifies that consequences of transferred hypoxia are strongly reflected in a condition of a liver which in late terms of reoxygenation is a source of MDA formation, supporting pathological process. Injection of a water-soluble antioxidant promotes considerable decrease in MDA level both in MC, and in MS fractions of liver since the 8th day. So, there is no authentic difference between indicators of MDA in the group receiving Fenozan and the control for 8th, 10th, 12th and 21st days of a life.

Changes of activity of enzymes of antioxidant protection of SOD and catalysis at the infant rats subjected pre-natal hypoxia, have unidirectional character, reflecting pressure of compensator abilities of antioxidant systems, and then their exhaustion and gradual restoration to which is promoted by Fenozan nanodoses.

Activity of SOD in brain tissues at infant rats of the experiment group at a birth is raised in 1.8 times concerning the control, by the end of the first days it sharply goes down to level in 1.9 times below the control. During 3-8 days the activity of SOD is gradually restored and, since the 10th day, authentically does not differ from the control. At Fenozan injection activity of SOD is restored much faster, becoming comparable with the control, since 5th day of a life that proves high efficiency of action of Fenozan in a brain tissue.

In blood the activity of SOD at rats of the experiment group is sharply lowered already at a birth - in 5.14 times concerning the control (authentically on Students t-test), and continues to decrease by the end of 1st day, becoming in 7.9 times lower, than in control group. Since 3rd day of experiment, activity of SOD increases a little, however does not reach control level even on the 21st day of a life, remaining lowered in 1.4 times in relation to it.

In MC fractions of a liver the activity of SOD at rats of the experiment group is authentically increased by the moment of a birth and sharply decreases (in 2.3 times) by the end of the 1th day of a life, remaining lowered in 1.8; 1.9; 2.5 times on 3rd, 5th, 8th days of their life (t-test data it is given in the Table 2). From 10th till 21st day of experiment activity of SOD at rats of the experiment group authentically lower the control in 2.2-2.3 times that specifies in stabile oppression of enzyme activity under the influence

of active forms of the oxygen which level of generation in these terms is increased. Fenozan injection promotes authentic increase in activity of SOD concerning this indicator at rats of the experiment group in all terms of supervision, however level of activity of SOD does not reach control sizes, authentically differing norms.

TABLE 2. SOD ACTIVITY (E/MG TISSUE PROTEIN) IN TISSUES OF NEONATAL RATS SUBJECTED PRE-NATAL HYPOXIA, EFFECT OF NANODOSES OF ANTIOXIDANT

				Brain homogenate				
Group of animals	At birth	24 hours	3rd day	5th day	8th day	10th day	12th day	21st day
Control (n=8-9)	0.53±0.04	0.58±0.05	0.57±0.07	0.72±0.05	0.95±0.03	1.11±0.06	1.31±0.11	1.36±0.09
Experiment (n=8-9)	0.96±0.07	0.31±0.02	0.36±0.02	0.47±0.03	0.72±0.06	1.06±0.07	1.37±0.07	1.32±0.07
Fenozan nanodoses (n=12-16)	-	0.34±0.02	0.36±0.03	0.64±0.03	0.89±0.02	1.12±0.08	1.11±0.03	1.18±0.05
P1:2	<0.001	<0.001	<0.05	<0.001	<0.01	>0.05	>0.05	>0.05
P 1:3	-	<0.001	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
P 2:3	-	>0.05	>0.05	<0.05	<0.05	>0.05	<0.05	>0.05
				Peripheral blood				
Control (n=8-9)	11.73±0.8	9.53±0.70	6.17±0.43	5.66±0.63	3.70±0.28	4.12±0.09	4.36±0.21	3.68±0.33
Experiment (n=8-9)	2.28±0.11	1.20±0.05	1.57±0.12	1.65±0.04	2.12±0.12	2.04±0.19	2.34±0.25	2.70±0.18
Fenozan nanodoses (n=12-16)	-	1.27±0.05	1.89±0.05	2.40±0.08	2.89±0.08	2.93±0.10	3.64±0.13	3.39±0.11
P1:2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
P 1:3	-	<0.001	<0.001	<0.001	<0.05	<0.05	<0.01	>0.05
P 2:3	-	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005
				Mitochondrial fraction of liver				
Control (n=8-9)	6.09±0.60	5.37±0.85	3.36±0.20	4.39±0.44	6.08±0.54	6.19±0.47	5.99±0.48	8.03±0.98
Experiment (n=8-9)	8.32±0.87	2.36±0.10	1.86±0.04	2.27±0.21	2.40±0.19	2.79±0.30	2.55±0.08	3.55±0.30
Fenozan nanodoses (n=12-16)	-	2.78±0.20	2.39±0.19	2.62±0.13	3.35±0.14	3.67±0.23	4.10±0.21	5.32±0.31
P1:2	<0.05	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P 1:3	-	<0.01	<0.01	<0.001	<0.001	<0.001	<0.01	<0.05
P 2:3	-	>0.05	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05
				Microsomal fraction of liver				
Control (n=8-9)	1.02±0.07	1.12±0.14	1.15±0.05	1.37±0.09	1.67±0.12	2.40±0.13	3.09±0.20	5.18±0.87
Experiment (n=8-9)	0.75±0.09	0.77±0.16	0.51±0.12	0.42±0.05	0.46±0.07	0.55±0.06	0.66±0.09	1.45±0.08
Fenozan nanodoses (n=12-16)	-	0.51±0.04	0.62±0.05	0.74±0.06	1.16±0.05	1.52±0.09	2.20±0.12	3.91±0.15
P1:2	<0.05	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P 1:3	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	>0.05
P 2:3	-	>0.05	>0.05	<0.01	<0.001	<0.001	<0.001	<0.001

In MS fraction of a liver the SOD activity is lowered in all terms of research in 2.3-4.3 times, and the peak of oppression of its activity is occurred on the 10th day (in 4.7 times), and on the 21st day of a life the activity of SOD keeps lowered in 3,5 times. At Fenozan injection the SOD activity also remains lowered, however intensity of fall makes 1.9-1.3 times concerning the control. On the 21st day of a life at Fenozan injection the SOD activity authentically is not differed from the control.

As a whole level of changes of SOD activity after chronic hypoxia - reoxygenation shows high compensator properties of a brain where spontaneous normalization of SOD activity occurs on the 10th day of a life, and high efficiency of Fenozan in a brain tissue since at its introduction the increase in activity of enzyme occurs in 2 times earlier - on the 5th day. In blood, and also in subcellular fractions of a liver the spontaneous normalization of

SOD activity does not occur in all terms of supervision, and Fenozan injection promotes increase of activity of the enzyme, the most appreciable in MS fraction where a full restoration of SOD activity is observed on the 21st day.

The analysis of catalase activity has shown, that in brain homogenate directly after a birth it is a little increased (in 1.2 times), and then sharply goes down, reaching values in 1.6 time slower than the control on the 3rd days of a life. By 5th day growth catalase activity, having not expressed character because on the 21st day of experiment activity of catalase keeps lowered in 1.5 times concerning the control is observed. At Fenozan injection the restoration of catalase activity is occurred in brain homogenate, since 8th day of a life. In spite of the fact that activity level of catalase at the rats receiving Fenozan thus lower than the control, but it authentically differs from indicators in the experiment group (t-test data it is given in the Table 3).

TABLE 3. CATALASE ACTIVITY (MMOL H_2O_2 / MG TISSUE PROTEIN X MIN) IN TISSUES OF NEONATAL RATS SUBJECTED PRE-NATAL HYPOXIA EFFECT OF NANODOSES OF ANTIOXIDANT

Group of animals	At birth	24 hours	3rd day	5th day	8th day	10th day	12th day	21st day
Brain homogenate								
Control (n=8-9)	22,0±1,0	21,7±0,5	23,6±0,3	25,4±0,3	28,1±0,5	30,4±0,5	31,1±0,5	29,8±0,5
Experiment (n=8-9)	26,3±0,4	16,0±0,7	15,2±0,3	17,1±0,6	16,9±0,4	18,0±0,7	17,0±0,4	20,1±0,8
Fenozan nanodoses (n=12-16)	-	17,1±0,6	18,4±0,6	19,9±0,4	25,5±0,5	25,9±0,5	26,4±0,5	27,0±0,3
P1:2	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
P 1:3	-	<0,001	<0,001	<0,001	<0,01	<0,001	<0,001	<0,001
P 2:3	-	>0,05	<0,001	<0,01	<0,001	<0,001	<0,001	<0,001
Peripheral blood								
Control (n=8-9)	31,9±0,6	32,0±0,6	31,1±0,7	36,4±0,3	36,8±0,3	37,0±0,3	38,0±0,4	37,9±0,3
Experiment (n=8-9)	42,1±0,5	32,7±0,8	19,9±0,5	24,1±0,5	29,2±0,8	30,0±0,6	34,1±0,4	35,0±0,9
Fenozan nanodoses (n=12-16)	-	31,2±0,5	23,0±0,6	28,9±0,8	32,9±0,6	32,9±0,5	35,0±0,3	36,0±0,3
P1:2	<0,001	>0,05	<0,001	<0,001	<0,001	<0,001	<0,001	<0,01
P 1:3	-	>0,05	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
P 2:3	-	>0,05	<0,001	<0,001	<0,001	<0,001	>0,05	>0,05
Mitochondrial fraction of liver								
Control (n=8-9)	58,7±0,8	61,2±0,4	60,9±0,9	63,2±0,6	63,4±0,5	62,4±0,6	63,0±0,4	63,3±0,3
Experiment (n=8-9)	78,4±0,3	56,3±0,6	50,6±0,6	50,8±0,9	57,1±0,5	55,2±0,5	58,4±0,3	59,3±0,7
Fenozan nanodoses (n=12-16)	-	56,0±0,5	56,2±0,5	58,1±0,5	61,2±0,8	60,0±0,6	61,0±0,6	61,0±0,5
P1:2	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
P 1:3	-	<0,001	<0,001	<0,001	<0,05	<0,05	<0,05	<0,001
P 2:3	-	>0,05	<0,001	<0,001	<0,001	<0,001	<0,001	>0,05
Microsomal fraction of liver								
Control (n=8-9)	20,1±0,6	21,1±0,5	21,1±0,3	21,5±0,5	22,0±0,2	23,1±0,3	22,2±0,4	22,1±0,4
Experiment (n=8-9)	24,0±0,7	17,8±0,4	17,0±0,3	17,5±0,5	17,9±0,4	18,4±0,4	18,8±0,6	18,1±0,8
Fenozan nanodoses (n=12-16)	-	17,2±0,3	17,1±0,4	19,1±0,6	21,0±0,4	21,1±0,6	21,0±0,6	22,0±0,5
P1:2	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
P 1:3	-	<0,001	<0,001	<0,01	<0,05	<0,01	>0,05	>0,05
P 2:3	-	>0,05	>0,05	<0,05	<0,001	<0,001	<0,05	<0,001

Dynamics of catalase activity in blood after chronic hypoxia - reoxygenation reflects reaction of a whole organism and testifies to mobilization of compensator - adaptive mechanisms by the moment of a birth with their subsequent exhaustion by 3rd day of a

life. Since 5th day, an increase of catalase activity in blood is observed which, however, does not reach control level. Fenozan injection promotes normalization of catalase activity in blood by 21st day of a life and its authentic increase concerning the experiment group in other terms of supervision.

In subcellular fractions of a liver there is a similar tendency of change of catalase activity though efficiency of Fenozan in MC and MS fractions is various. Authentically influence of Fenozan in MS liver fractions where catalase activity is restored to norm by 12^{th} day is more effective. In MC fractions at Fenozan injection the restoration of catalase activity to norm occurs only on the 21st day of a life.

Analyzing dynamics of catalase activity as a whole, we should notice that spontaneous restoration of activity of enzyme to norm by 21st day of a life does not occur in one of the studied tissues. Thus by the birth moment in all tissues the increase in activity of enzyme, and then its sharp recession is observed. The oppression of catalase activity on the 1^{th} day is more expressed in a brain tissue (in 1.3 times), is absent in blood and MC liver fractions, is poorly expressed in MS fraction (1.2 times) concerning the control. The peak of suppression of catalase activity in a brain and blood corresponds to the 3rd day, in MC - to 5th day, in MS - to 3rd day. It is remarkable, that in MC and MS throughout 3-8 days catalase activity does not change almost in dynamics, keeping lowered in 1.2 times in comparison with the control.

Fenozan injection makes positive impact, promoting increase of catalase activity in all tissues though its full normalization to control level is observed only in MS liver fractions on the 12th days of a life. In a brain, blood and MC fractions of a liver the catalase activity raises, authentically differing from indicators in the experiment group, but does not reach control sizes.

As have shown results of our researches, the most vulnerable are rats on the 3-8^{th} days of a life concerning intensity of generation of active forms of oxygen. If by 10^{th} day a level of generation ROS in a tissue decreases, by 21st day there is a full normalization of concentration MDA. It will be coordinated with data Gabriel G. Haddad, 2009 which have established, that at rats for 4-7th day of a life sensitivity to excitotoxic amino acids is minimum and increases by 8-12th day, and on the 18th day from NMDA excitotoxicity 90% neurons are died (Panchman, 2008).

In a brain the activation of reactive oxygen species (ROS) generation in MC immature neurons is predictor of calcium exit and start of the NMDA-toxic cascade; it is also predictor of membranous potential failure with development of disorder of absorption of oxygen by a cell and its destruction. The neurons are capable to stop broken calcium homeostasis (if it is short and lasts some days); if disorder lasts more than week, then it is impossible and cells are died. We believe, that ultra low doses of an antioxidant Fenozan helps to stop activation of ROS generation during these critical periods and to provide normal development of brain tissue cells.

The received results are confirmed with earlier revealed fundamental representations about mechanisms of Fenozan effects and their dependence on a dose. The Fenozan possesses stabilizing effect on the superficial layers and deep layers of lipids of plasmatic membrane and membranes of endoplasmic net in a wide range of concentration 10^{-3}-10^{-23} M (Burlakova, 2010).

The great Fenozan concentrations - 10^{-3}-10^{-5}M - considerably change structure of membranes, destruct microdomains of lipids therefore, protein-lipid domains are re-formed too. Thus, in concentrations 10^{-6}-10^{-14}M its effects practically are not expressed – "a dead zone". Maxima on curves on change of structural characteristics of lipids under action of Fenozan coincide with a maximum of their biological activity in vivo. The ultralow concentrations of Fenozan - 10^{-18}-10^{-19}M change microviscosity of deep-laying lipids, and order of superficial lipids; induce occurrence of additional structural transition in the range of physiological temperatures that can lead to changes in work of membrane-connecting enzymes. The Fenozan in ULD is the super activator of Protein kinase C enzyme (PKC). It is proved by qualitative correlation between change of microviscosity of

a plasmatic membrane and activity PKC that is possibly caused by complex formation of Fenozan with PKC or embedding of Fenozan into the special membranous clusters, containing PKC. The Fenozan operates on a plasmatic membrane in 2 times higher, than on microsomal membranes (Alekseeva et al., 2010).

There are data, that effects Fenozan are caused by its direct influence on enzymes, reaction with peroxy radicals, reaction with ROS, receptor interactions, a parametrical resonance, structural memory of water that is especially brightly shown at action of pretended concentration of Fenozan 10^{-19}-10^{-23}M. According to Andrievskiy (2008) "there can not be more universal antioxidant or regulator of free-radical processes, than the water structures with specific order". It is established, that only water molecules are capable to form clusters around molecules of biologically active substance. BAS in ULD has effect on structure of this boundary water forming a layer with thickness of tens and hundreds microns. The properties of water in it are so distinct from volume water, that boundary water should be considered as a special modular phase of liquid water. Specificity of this water is the mobile excited condition of electrons therefore this water possesses electron-acceptor properties. An example of formation of such structured hydrophilic membrane is "the ordered cluster water" around hydrated fullerenes. The hydrated fullerenes are harmless and show only positive biologic activity, possess anti-neurodegenerative, radioprotector, antitoxic, anti-oxidant properties. Probably the studied fall of concentration of an end-product of MDA lipoperoxidation under Fenozan effect is caused by decrease in its formation due to breakage of a chain as a result of self-neutralization reaction (recombination) of free radicals that can be in turn caused by properties of special structures of the water ordered by Fenozan.

Conclusion

ROS have been shown to play important roles as both cellular messenger molecules in physiological events, such as activity-dependent synaptic plasticity and memory, and toxic molecules in choric hypoxia. As we have established, chronic pre-natal hypoxia is accompanied by accumulation of the end-product of lipoperoxidation - MDA in brain tissue, blood and subcellular fractions of a liver, with the subsequent spontaneous normalization of its maintenance by 21st day in a brain and blood also its absence in a liver that shows the support of pathological process. It is prospective that ROS generation in the subcellular fractions of the liver in the late period after hypoxia took part in persistence of pathological process and late neuronal cells death. Because ROS are both beneficial and deleterious to neuronal function, the balance between ROS formation and antioxidant enzymes is critical for normal neuronal function. Fenozan injection in ULD leads to appreciable decrease the ROS generation without influence to its minimal level, which is necessary to neuronal plasticity. The greatest efficiency of Fenozan concerning restoration of enzyme activity of SOD and catalase is marked in MS liver fractions where normalization of SOD occurs on the 21st day and catalase - on the 12th day. Normalization of the ROS scavenger enzymes activity (SOD and catalase) in the liver specifies that Fenozan in ULD has positive effect to oxidative stress in the late period after chronic intrauterine hypoxia and can provide precondition for normalization of pathological process in earlier terms, accompanied with neuroprotection. Significance of this data support that ultra low doses of Fenozan can be less invasive and effective in the treatment of chronic intrauterine hypoxia and suggest the directions for further research.

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