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IL-1ra stabilizes the thiol-disulfide system in the brain tissues of rats with experimental diabetes

Abstract: Effect of piracetam, thiocetam and interleukin-1 receptor antagonist (IL-1 ra) (7.5 mg/kg) on the values of thiol-disulfide system (TDS) and protein oxidative modification (POM) was studied based on the rat alloxan diabetes model. It is established that post-ischemic damage to brain tissue of the experimental animals was followed by multidirectional thiol-disulfide imbalance (increase in levels of oxidized forms of glutathione and thiols on the background of sharp decrease in their reduced forms), decreased activity of TDS enzymes (glutathione peroxidase and glutathione reductase) and increased level of POM markers - APhH and KhH. It is proved that course introduction of piracetam, thiocetam and IL-1ra was beneficial in stabilizing TDS and POM values, normalizing activity of glutathione peroxidase and glutathione reductase, with maximum activity noted for IL-1ra.

Keywords: interleukin-1, IL-1 ra, experimental diabetes mellitus, thiol-disulfide system.

Diabetes mellitus (DM) is one of 7 main mortality causes in most countries of the world and is third among immediate causes of death after cardiovascular and oncological diseases [1]. In Ukraine, a steady increase of DM prevalence is noted - a number of diabetic patients has increased more than by half for the past 10 years and now makes up nearly 1 million people. Therefore solution to the DM therapy problems is still pressing medical and social issue. [2]. Nowadays, a body of evidence is amassed in the whole world, as to the fact that effective DM control determines life expectancy of the patients and their performance capability, as well as may keep development of the related complications to a minimum.

High incidence rate of DM complications is determined by tissue metabolism with the damage to microcapillary organ bed, which causes multi-organ pathology. In case of DM, insulin deficiency causes carbohydrate, fat and protein metabolism disorder, trigger hyperglycemia, insulin resistance and energy deficiency, activation of synthesis of reactive oxygen intermediates, free radicals and products of lipid peroxidation, i.e. creates pathophysiological picture of the oxidant stress. In its turn, oxidant stress causes increase in leukocyte adhesion, platelet aggregation and generation of endothelial dysfunction. Metabolic diabetic endothelial dysfunction is a release mechanism for development of angiopathies, including neurological complications of DM: micro- and macroangiopathies, encephalopathies, cerebrovascular disorders, distal neuropathies [3]. Furthermore, vascular changes induce ischemic/hypoxic tissue disorders, and metabolic disorders cause immediate damage and death of cell structures of the central nervous system and peripheral nervous system. This leads to the changes of functional metabolic state of cells, which are determined by generation of mitochondrial dysfunction, energy deficiency and development of the bioenergetic (tissue) hypoxia [4]. Therefore, due to DM metabolic and vascular disorders mutually potentiate their own pathophysiological effects and complete the vicious circle of multi-system organ damage - processes of vascular wall and highly metabolic tissue damage worsen, which ensures early development of the neurological complications. Thus, primary goal of the effective DM therapy is to block interdependent mechanisms of DM progression - vascular, metabolic events and oxidant stress phenomenon, for which reason increasingly greater attention is paid to medications with antioxidant effect.

It is proved, that the most important biological roie for antioxidant system is played by reduction-oxidation reactions, during which thiol groups are easily oxidized, generally to form disulfide groupings, and again are regenerated under their reductive splitting. Based on such transformations, a reversible thiol-disulfide system (TDS) is formed. TDS intermediates exhibit transport properties as to nitrogen oxide (NO), thus increasing its bioavailability. Besides, many thiols - glutathione, cysteine, methionine - are able to considerably limit cytotoxicity of NO and its derivatives, thus multiplying neuron chances to survive in case of ischemia [5].

Agents, providing both for damaging effect and cell viability system in the ischemia/hypoxia area, include cytokines - intercellular communication transmitters in helth and diasease which estabish comunication sinnal networkbetween cells

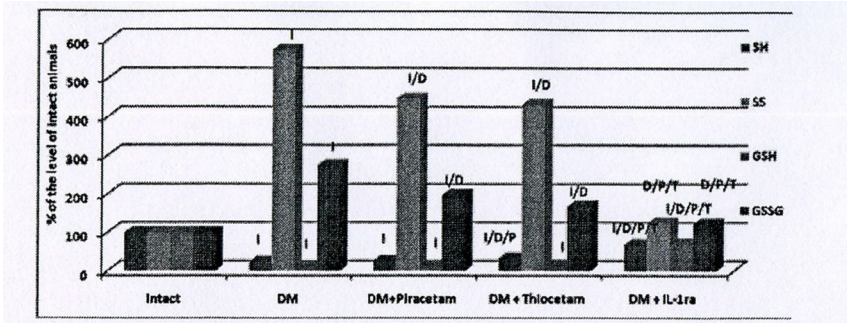

Fig. 1 Values of reduced (SH) and oxidized (SS) thiols, and reduced (GSH) and oxidized (OSSG) glutathione in the brain tissue of DM rats

Notes for Fig. 1-4: Intact - intact rats; DM - diabetes mellitus; DM+Piracetam - diabetes mellitus + Piracetam; DM + Thiocetam - diabetes mellitus + Thocetam; DM + IL-1ra - diabetes mellitus + IL-1ra. Statistically significant differences ($p<0,05$) for intact rates are marked with "I", for rates with diabetes mellitus - with "D", for rats from DM+Piracetam group - with "P", for rates from DM + Thiocetam group - with "T".

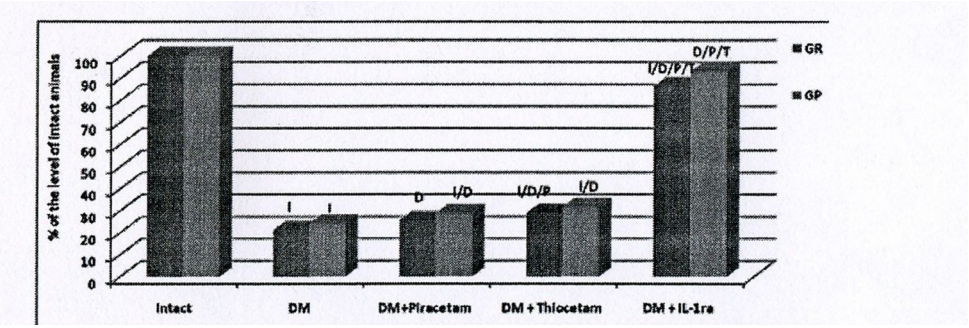

Fig. 2. Activity of glutathione reductase (GR) and glutathione peroxidase (GP) in the DM rat brain tissue

Also, development of DM in rats was followed by decrease in cysteine and methionine levels as compared to the intact group by 64% ($p<0,001$) and 60% ($p<0,001$) respectively on the background of sharp increase in the levels of potentially neurotoxic substances - homocysteine (by 5.1 times, $p<0,001$) and nitrotyrosine (by 6 times, $p<0,001$), which proves development of oxidative and nitrosating stress in the brain tissue of rats with experimental DM (Fig. 3).

Under conditions of the experimental therapy, the following results of piracetam, thiocetam, and IL-1ra effect on thiol-disulfide balance (Fig. 1-3) were obtained. On the background of piracetam administration, a certain stabilization in the DM rat brain tissues was noticed for the studied TDS activity values as compared to the control animals - levels of oxidized thiols and glutathione were lowered by 22% and 27% respectively ($p<0,001$), changes in the rest of values were expressed in a lesser degree.

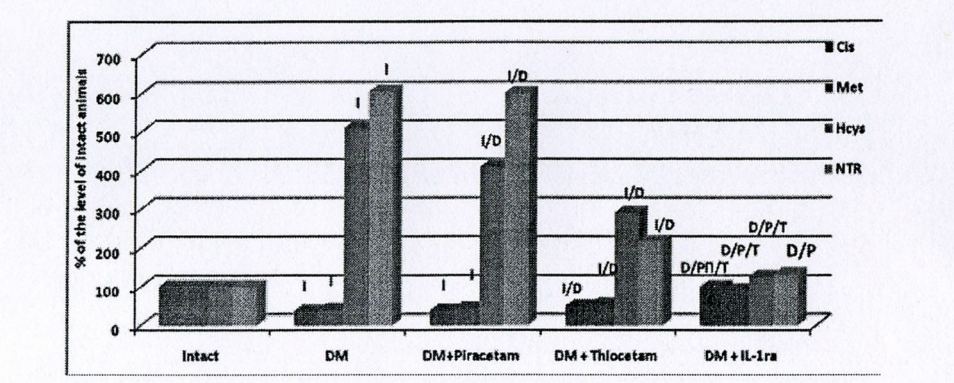

Fig. 3. Content of cysteine (Cys), methionine (Met), homocysteine (Hcys) and nitrotyrosine (NTR) in the brain tissue of DM rats

On the background of thiocetam administration, oxidized glutathione formation is blocked in the experimental animals by 25% ($p<0,01$) and formation of oxidized thiols is blocked by 39% ($p<0,001$) on the background of elevation of their reduced forms - by 27% and 43% respectively ($p<0,01$) - and increase in GR and GP activity by 32-37% as compared to control values.

Administration of IL-1ra to the DM animals had the most expressed affect on TDS state - levels of oxidized thiols and glutathione were decreased by 55% ($p<0,001$) and 78% ($p<0,001$) respectively as compared to control values. In this context, levels of reduced glutathione and thiols are actively increased (to 70% of the intact values) and activity of TDS enzymes is restored - during acute period of

diabetes activity of GR and GP is increased almost fourfold (p<0,001). Furthermore, use of IL-1 ra resulted in almost equivalent decrease in the levels of neurotoxic homocystein and nitrotyrosine in the brain tissue by 75% (p<0,001) and 78% (p<0,001) respectively as compared to control values, with cystein and methionine levels being restored nearly to the intact animal measures. Similar dynamic for such values, yet less expressed, was noticed for the animals from Thiocetam group.

Ischemic damage to brain tissue of the experimental animals with experimental DM was followed by increase of POM markers in the brain homogenate - aldehyde phenylhydrazones (APhH) and ketone phenylhydrazones (KPhH), which are formed under conditions of oxidative and nitrosating stress (Fig. 4).

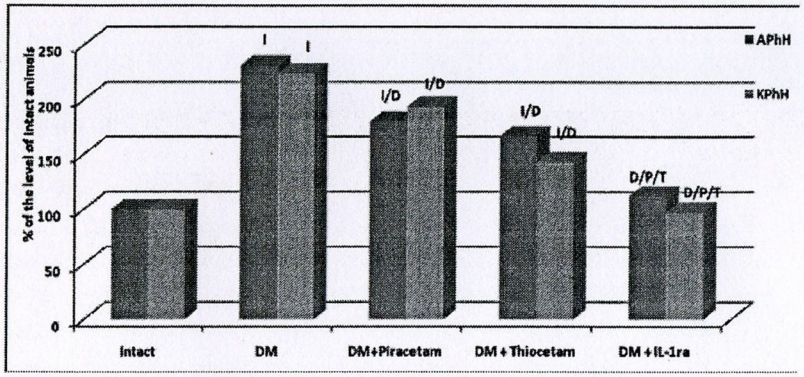

Fig. 4. Content of aldehyde (APhH) and carboxyle (KPhH) products in the DM rat brain tissue

In our research, development of alloxan diabetes was followed by the expressed elevation of APhH and KPhH levels – by 131% (p<0,001) and 124% (p<0,001) respectively. Use of piracetam and thiocetam resulted in significant decrease in POM markers, which is more expressed in the thiocetam group (decrease of KphH by 36% (p<0,001)). Course administration of IL-1ra facilitated the most significant stabilization of protein oxidative modification and almost twofold decrease (p<0,001) in its markers in the brain tissue, with KphH nearly reaching intact animal measures.

Therefore, in case of ischemic dmaage to the brain tissue in DM model, TDS balance shifts due to decrease in its reduced intermediates on the background of oxidized forms, with considerable lowering of reduced glutathione level and GR and GP activity. Similar pathological biochemical changes cause significant functional changes in cells and are often irreversible. Changes on TDS activity and oxidation of thiol groups of a cystein-dependent protein region of the mitochondrial internal membranes cause depolarization and destabilization of the mitochondrial internal membranes, with so called non-selective PT-pore (permeability transition pore - PTP) being formed [12]. Opening of such channel in the internal membrane results in establishing ion balance in the matrix and mitochondrial intermembranous space, distributes hydrogen ion gradient (H^+) to the internal membrane and breaks respiratory chain. Also, this causes volume disregulation of mitochondria due to the matrix hyperosmolality, results in the increased matrix volume, breaks of the external membrane and growing destabilization of mitochondria and enzyme system, leads to development of persistent mitochondrial dysfunction, and as a result, to mitochondrial death - mitoptosis. Moreover, IL-1, produced in response to hypoxia, expresses inducible NOS (iNOS) in the glial cells, which results in NO hyperproduction and toxic effects die to its excessive amount. Excessive amount and its highly toxic derivatives nitrosylate protein-clinging enzymes of the respiratory chain of mitochondria and Krebs cycle, and inhibit them [13]. Dysfunction of mitochondrial enzyme complexes (MEC) is formed, which causes qualitative changes of iron-sulfur centers in the mitochondrial enzymes and their functions, as well as suppression of a main (NAD-dependent) pathway for the substrate oxidation in respiratory chain. Aerobic energy synthesis is suppressed, thus bioenergetic (tissue) hypoxia is developed [14]. Under conditions of the impaired generation of cell energy, caused by mitochondrial dysfunction, loss of NAD and ATP results in death of cells by necrosis or apoptosis [15]. These pathophysiological changes form the basis of occurrence of early or late post-ischemic DM complications, resulting in disturbance of the usual lifestyle and lowering of life quality, persistent loss of occupational capacity and rapid progression of heavy neurological consequences up to lethal outcome.

To gain maximum protective effect in the DM therapy, it is necessary to achieve interruption of pathogenetic ischemic/hypoxic cascade at earlier stages, which includes stage of thiol-disulfide imbalance establishing. Normalization of TDS state allows prevention of depolarization and destabilization of the mitochondrial

internal membrane followed by development of mitochondrial dysfunction, energy imbalance and other post-ischemic consequences.

Conclusions

1. Development of alloxan diabetes with onset of post-hypoxic tissue changes was followed by the discordant shifts of thiol-disulfide system components ((increase in levels of oxidized forms of glutathione and thiols on the background of sharp decrease in their reduced forms), decreased activity of TDS enzymes, as well as increased content of neurotoxic homocystein and nitrotyrosine and markers of protein oxidative modification in the rat brain.

2. Course administration of piracetam, thiocetam, and IL-1ra facilitated stabilization of thiol-disulfide balance and decreased activity of free radical oxidation reactions in the DM rat brains.

3. IL-1ra activity toward normalization of thiol-disulfide system and inhibition of manifestations of oxidative and nitrosylate tress is higher than similar activities of piracetam and thiocetam.

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