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ANALYSIS OF GLUTATHIONE ENZYME ACTIVITY AND INDICATORS OF OXIDATIVE MODIFICATION OF LONG LIVERS' BLOOD SERUM PROTEINS (CARPATHIAN REGION)

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ABSTRACT

The analysis of the functional state of the enzymatic detoxification of xenobiotics and oxidative modification of proteins in the serum of 60 long livers (study group) and 30 individuals having no long livers in their family trees (control group) was done. It was found that the activity of glutathione peroxidase in all long livers was respectively (0.329 ± 0.18) mmol / (min • mg) (study group), and (0.353 ± 0.17) mmol / (min • mg) (control group). The activity of the enzyme glutathione reductase in long livers (Carpathian region) was 3.17 times higher compared with persons of control group. Tendency to decreased GST activity respectively in long livers (0.305 ± 0.31) mmol / (min • mg) and control group (0.345 ± 0.18) mmol / (min • mg) was found out. The significant reduction of oxidative modification of protein products of neutral aldehyde and ketone derivatives in study group compared with control one was proved statistically. The intensification of the processes of oxidative modification of proteins in long livers was accompanied by an increase by means of aldehyde and ketone derivatives compared with persons of mature age. These results may indicate better functioning of antiradical protection of long livers compared with persons of old age.

Key words: Long livers, Protein oxidative modifications, Glutathione peroxidase, Glutathione reductase, Glutathione-S-tranferase.

INTRODUCTION

The revolutionary view on the molecular mechanisms of living systems is offered by young dynamic science called epigenetics [1]. According to prof. A. M. Vaiserman, epigenetics, the branch of genetics, became as an independent field of study not long ago. One of the most inspiring epigenetic hypothesis, proved that the activity of many genes susceptible to outside influence, is confirmed in experiments on model animals nowadays. Life longevity is known to be a multifactorial trait, and thus both hereditary and external factors have influence on its expression. Quite often they have a negative effect.

In today's anthropogenic capacity the study of the functioning detoxification system is becoming relevant. Biotransformation process involving enzymatic conversion of foreign inclusions or xenobiotics, is divided into three phases [2-5]. Phase I of activation of xenobiotics and metabolic transformation is based on joining of modifying functional groups (-OH ,-SH,-NH2) to them. While oxidation, recovery and hydrolysis taking place there, the formation of intermediate metabolites are formed. This process is catalyzed by the microsomal enzymatic system of cytochrome P450 (cytochrome enzyme family) and some other classes of enzymes oxidase, reductase, hydrolase and dehydrogenase. During phase II of

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biotransformation – neutralization, the intermediate metabolites are combined with endogenous ligands, increasing the hydrophilic nature of these compounds, thereby contributing to their removal from the body. That is phase II is the conjugation of hydrophilic macromolecular substances with different substrates, and as a result they are converted into hydrophilic conjugates capable for expression in the bile. Phase III is to evacuate or derive soluble non-toxic substances from the body. To do this, there are specific vectors of exogenous compounds – P-glycoproteins promoting excretion of xenobiotics in the bile or blood.

It is known that any adaptive or pathological process runs on the background of the formation of reactive oxygen species [6,7]. Under conditions of oxidative stress, processes of protein peroxidation are running very active, ultimately leading to the loss of their biological activity, while oxidation modified proteins are generating new antigens and have negative impact on the immune response [8, 9]. Active oxygen species cause oxidative modification of proteins (OMP) under normal and pathological conditions. For normal functioning of the body the dynamic balance between prooxidant and antioxidant systems is supported. Oxidation of proteins is a normal body functional process, associated with vital functions. Moreover, the latter are largely interrelated with the protective and adaptive reactions of the organism, namely in the biotransformation of xenobiotics. Increased OMP product level is the result of an imbalance between the processes regulating protein synthesis and oxidation, and reduction of protease activity cleaving selectively oxidized forms of proteins. OMP may comprise direct protein fragmentation or cause their denaturation with partial or complete loss of function [10]. These changes lead to a decrease in adaptation processes of the organism as a whole, causing the development of pathological conditions. Animal testings have shown that aging is accompanied by accumulation of oxidative protein modification in specific tissues [11].

As a result, our previous studies found the deletion allele of GSTT1 and GSTM1 gene associations with life longevity. As for functional genomics it is of vital importance to determine the activity of enzyme systems of xenobiotic biotransformation, since the existence of functional differences between alleles within a locus determine allelic differentiation in the expression protein level, efficiency of the transport function, activity, thermostable enzymes, immune response and so on. Special interest is paid to study these processes in long livers.

Aim of the research to study the relationship between enzymatic activity of glutathione system, indicators of oxidative protein modification in blood plasma, and life expectancy among long livers (Carpathian region).

MATERIALS AND METHODS

The functional state of the enzyme system and detoxification of xenobiotics and antioxidant protection was studied taking into account the indicators of serum – study group (60 long livers) and control group (30 mature individuals), having no long livers in their family trees.

The activity of GST was assessed the rate of formation of glutathione-S-conjugates between glutathione and 1-chloro-2,4-dinitrobenzene [12]. The activity of glutathione reductase (GRD) was determined by the rate of change of optical density (340 nm) caused by the oxidation of NADP • H [13], glutathione peroxidase (GPO) – from the reaction of reduced glutathione with tret- butyl hydroperoxide [14]. OMP products in serum were investigated using O.Dubinin method [15] based on the interaction of oxidized amino acid residue protein with 2.4 dinitrophenylhydrazyne (2.4 DNPH). OMP degree was evaluated taking the amount of aldehyde and protein ketone derivatives of neutral and basic character. All samples were done by spectrophotometer of wavelengths 356, 370, 430 and 530 Nm. For statistical data analysis software Microsoft Excel was used.

RESULTS AND DISCUSSION

As a result of metabolic transformation of substances in the body, free radicals are formed having high chemical activity, causing lipid, protein, nucleic acid peroxidation processes [16]. Having formed in the body, they interact with the cell structures, leading to the cell damage, mostly membrane, thus causing the development of pathological process. The damaging effect of free radicals is reduced by enzymes, providing the antioxidative protection. Glutathione system is considered to be powerful antioxidant.

Having investigated the enzymes of glutathione system it was found that GPO activity (study group, long livers) was 0,329 ± 0,18 mmol / (min • mg), and control group 0,353 ± 0,17 mmol / (min • mg) respectively (Table). Glutathione peroxidase is an enzyme involved in the hydrogen peroxide and organic peroxides inactivation in the cells of higher animals and humans. GPO- glycoprotein has four atoms of selenium in its active center. It is a hydrophilic compound, which makes it difficult to penetrate into the lipid layer of the membrane, its basic part is localized in the cytosol and another – in the mitochondria. GPO has selenium isoenzymes: extracellular GPO, found in plasma and milk, GPO-G1, isolated from the cytosol of liver cells and intestinal isoenzyme and non-selenium isoenzyme identical to GST.

The results of the functional activity of GPO genes in mice have shown that in knockout variant only one allelic gene of glutathione peroxidase have a normal phenotype, normal life expectancy [17]. These data prove that this enzyme is not critical for life activity. However, knockout mice having two copies of the gene, have early cataracts and defects causing muscle cell proliferation are observed. However, knockout mice having GPO-G4 gene

(glutathione peroxidase – 4) die during early embryonic development. There was a report that the reduced level of glutathione – 4 can increase life duration in mice [18]. GPO activity in living cells is increased by ionizing radiation, ethanol intoxication, acrylonitrile, and E- avitaminosis. The role of GPO is especially important under oxidative stress as prevents the nascence and development of peroxidation processes. GPO is one of the most important components of the antioxidant enzyme system.

In reactions catalyzed by GPO, oxidized glutathione (GSSG) is formed, to restore it in the cells there is a special enzyme – glutathione reductase [4]. Glutathione-S-transferase is equally important in the system of detoxification of xenobiotics and prooxidant activity reduction. The basic function of GST is the protecting of cells against xenobiotics and lipid peroxidation products through their recovery, joining them to the substrate molecule glutathione or nucleophilic substitution of hydrophobic groups. Our studies showed that GRD and GST activity in long livers were (0.219 ± 0.12) and (0.305 ± 0.31) mmol / (min • mg) (see table) in control group respectively – (0.069 ± 0.05) and (0.345 ± 0.18) mmol / (min • mg). Thus, the taken results showed that at almost the same GST activity in both groups, study and control ones, the activity of the GRD enzyme in long livers was 3.17 times higher compared with those of mature age.

After examining the level of plasma of OMP products in long livers, we found the decrease ($p < 0.05$) of neutral aldehyde and ketone derivatives with maximum absorption at a wavelength of 356 and 370 Nm (1.142 ± 0.050), (1.048 ± 0.035) and (1.414 ± 0.176), (1.246 ± 0. 098), (control group) (Fig. 1).

This trend may prove about better synthesis regulation and less protein oxidation in longevity, and increased activity of proteases cleaving selectively oxidized forms of proteins. Taking into account that study and control groups were in the same environmental conditions less OMP intensification of neutral aldehyde and ketone derivatives in long livers may indicate better functioning of antiradical defense systems.

The research of basic aldehyde and ketone derivatives showed that long livers have slightly higher levels of these products in the blood plasma compared with persons of old age, but these differences were not statistically significant ($p > 0.05$).

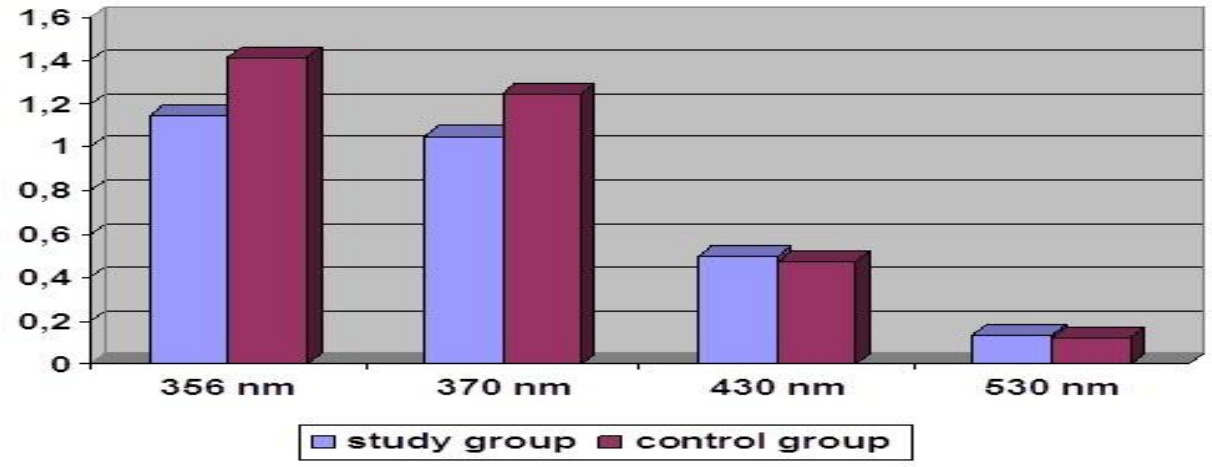
Study of influence of various toxic compounds on experimental animals according to the literature [10] indicates changes in the level of basic aldehyde and ketone derivatives, while it is mostly noted that deviation 2,4 DNF basic hydrazons is less developed.

Several studies have analyzed the role of molecular markers associated with the speed of the aging process [19-24]. In particular, instead of the oxidative stress theory a versatile "green theory of aging" is proposed. According to the latter, aging is considered as a result of macromolecular disbalance caused by the action of various endogenous and exogenous substances and toxic metabolic products, including the influence of oxidative stress and free radicals, and life expectancy is determined by the rate at which toxic substances are removed from the body, and by efficiency of damage correction.

Table 1. The Enzyme Activity of Glutathione System in Long Livers (Carpathian Region), M ± m

Groups	Enzyme Activity, mmol /(min·mg)		
	GPO	GRD	GST
Study group, n = 60	0.329±0.18	0.219±0.12*	0.305±0.31
Control group, n = 30	0.353±0.17	0.069±0.05	0.345±0.18

Figure 1. Indicators of oxidative protein modification (study and control groups)



CONCLUSION

It was found that GPO activity in all long livers was (0.329 ± 0.18) mmol / (min • mg), and control group (0.353 ± 0.17) mmol / (min • mg). It is shown that the activity of GRD enzyme in long livers (Carpathian region) is 3.17 times higher in comparison with those of mature age. It is found a declining trend of GST activity in long livers (0.305 ± 0.31) mmol / (min • mg) compared with control group (0.345 ± 0.18) mmol / (min • mg). It was diagnosed statistically the significant reduction in food OMP products of neutral aldehyde and ketone derivatives in study group compared with control one. Intensification of OMP processes in long livers was accompanied by the increase in basic aldehyde and ketone derivatives compared with persons of old age. These results may indicate better functioning of antiradical protection of long livers compared to old people.

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