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STUDING OF THE GLUTATHIONE SYSTEM ACTIVITY IN ASCITIC OVARIAN TUMOR UNDER EXPERIMENTAL CONDITIONS WITH GAMMAGLOBULINS TREATMENT

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Abstract

The article presents the experimental results of the investigation the glutathione system activity in rats simulated ovarian ascites tumor. It was found that the glutathione system in the form of glutathione reductase and glutathione peroxidase is inactivated after 2 months, but at 4 months of study the activity of these enzymatic systems increases. Treatment with gammaglobulins gives a certain therapeutic effect due to a change in the activity of the studied enzymes involved in redox intracellular reactions. It was established that for 2 months activation of glutathione peroxidase with parallel inhibition of glutathione reductase. However, for 4 months the activity changed in the opposite direction.

Keywords: *ascitic ovarian tumor, glutathione system, reductase, peroxidase, control pathology*

Introduction

Ascitic ovarian tumor is characterized by increased metabolism by catabolism processes. As a result, a large number of free radicals are formed. Many authors have already studied the question of the passage of catabolism in neoplasia cells. There are many works on the utilization of free radicals in the tumor and in the body as a whole [2, 5].

This question is important in a case of detoxification therapy for the utilization of free radicals. The problem becomes relevant after the appointment of chemotherapy regimens, the number of free radicals increases sharply due to the decay of tumors in the body [4].

The glutathione system has been not enough studied in oncological practice. The glutathione molecule is synthesized in the body. Therefore, endogenous and exogenous delivery routes are an advantage over other antioxidant systems. Also, in redox reactions, it can act as co-enzymes for the reduction of substances in biotransformation; independently acts as a stabilizer of the thiol status of protein structures [7].

The activity of the glutathione system can be studied in many ways, but the determination of enzymes for the synthesis of glutathione is the most favorable.

The aim of the study was to investigate the amount of glutathione reductase and glutathione peroxidase in rats simulated ovarian ascites tumor.

Methods

Experimental studies were performed on 20 white Wistar rats weighing 240-280 g, which were divided into 2 experimental groups (10 animals in each group): group 1 – control pathology – animals, which intraperitoneally atypical cells were administered. The cells in their histological composition corresponded to the ascitic ovaries tumor. This model is one of the classic models of carcinogenesis [2]; group 2 – control pathology with treatment by gammaglobulins.

Cell strains were selected from the bank of atypical cells of the N. N. Blokhin Oncology Center. This cell culture has already been used by various authors to obtain carcinogenesis under experimental conditions.

The manufacturers noted that the tumor had a histological picture of papillary adenocarcinoma, and metastases also corresponded in nature to adenocarcinoma. The method of obtaining cell selection is described by the authors to derive a pure line of atypical cells in our conditions. In the experimental conditions, we clearly adhered to all the parameters set by the authors [2].

After histological confirmation of carcinogenesis in our laboratory, we measured the amount of glutathione reductase and glutathione peroxidase by conventional methods. Then intraperitoneally injected ascitic fluid according to the specified method to animals included in the control and experimental groups [2].

The study group was administered specific immunoglobulins according to the new schemes of administration and dosing.

During the work with animals we complied with the International Code of Medical Ethics (Venice, 1983), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the General Ethical Principles for Animal Experiments adopted by the First National Congress of Bioethics (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes.

Statistical processing of the obtained results was performed using the program "Statistica 8.0". The probability of differences between the indicators of the control and experimental groups was determined by the criteria of Student and Fisher. The level of reliability was taken at $p < 0,05$.

Results

The concentration of glutathione reductase did not differ in groups of animals. In the group of control pathology without treatment, the value was increased on 0.005 U/l (Fig. 1).

On the second month in the group with control pathology without treatment there was a decrease in enzyme activity on 0.006 U/l (7.3 %). After 4 months of the study, the value increases on 0.014 U/l (18.4 %). Although during the study period, the value of glutathione reductase activity increased on 0.008 U/l (9.8 %).

In the treatment group animals after 2 months, the value of glutathione reductase activity

decreased on 0.048 U/l (55.2 %). Subsequently, at 4 months, the activity of the enzyme increased on 0.014 U/l (35.9 %). Thus, during the study period, the value of glutathione reductase activity decreased on 0.034 U/l (39.1 %.)

Was established the glutathione reductase activity decrease for 2 months in both groups. Moreover, in the group of control pathology there is decrease on 7.3 %, and in the treatment group on 55.2 %. For 4 months, synchronously in both groups there is an increase in enzyme activity on 18.4 % and 35.9 %, respectively.

In conclusion, it is observed that for 4 months of the study, the activity of glutathione reductase in the control pathology group increased compared to previous data on 0.008 U/l (9.8 %). Then in the treatment group, there is decrease in activity on 0.034 U/l (39.1 %).

At the beginning of the study, there were also differences in glutathione peroxidase activity between the control pathology group and treatment group of 0.027 U/l (5.7 %) (Fig. 2).

After 2 months, there was a slight decrease in glutathione peroxidase activity in the control pathology group on 0.003 U/l (0.6 %). Subsequently, there was a tendency to increase the activity of the enzyme in the treatment group on 0.041 U/l (8.6 %). In general, during the study period, the activity of the enzyme in the control pathology group increased on 0.037 U/l (7.6 %).

In the treatment group, for 2 months the values increased on 0.118 U/l (23.4 %). Subsequently, there was a tendency to decrease on 0.243 U/l (48.2 %). In general, in the study period, the value of glutathione peroxidase activity decreased on 0.125 U/l (24.8 %).

The study was disproportionately altered with glutathione peroxidase activity. In the control pathology group, initially there was slight decrease in activity on 0.6 %, and then the activity increased on 8.6 %. Accordingly, during the study period, activity increased on 7.6 %. At the same time, in the treatment group for 2 months the activity of the enzyme increased on 23.4 %, then after 4 months the activity decreased on 48.2 %. In general, during the study period with the use of gammaglobulins, the activity decreased on 24.8 %.

The initial data also show differences in the activity of enzymes in ascitic ovarian tumors. Thus, the activity of glutathione peroxidase is much higher than glutathione reductase.

Therefore, in view of the above, we began to compare the ratios of values to understand the activity of the enzymatic system. At the beginning of the study, we took all values as 0 points, in order to integrate indicators in one system (Fig. 3).

In the control groups, i.e. approximately in the group of control pathology there is decrease in the activity of both enzymes. Moreover, the activity of glutathione peroxidase is more inhibited. After 4 months, the value of this enzyme increases much more actively compared to glutathione reductase on 9.8 %.

Figure 3 shows a cross-reaction to the use of gammaglobulins. Thus, after 2 months, the glutathione reductase reaction is inhibited, although glutathione peroxidase is activated, the gap between them will be an incredible 78.6 %. In 2 months, there is turn in the way of inhibition of glutathione peroxidase to 48.2 %, from the initial level, and activation of glutathione reductase to 35.9 %. After 4 months of the study, the gap between the activities of enzymes is 84.1 %.

Thus, the activities of the enzymes in the study using gammaglobulins will intersect at the stages of the experiment at 2 and 4 months. The difference between these intersections is 5.5 %.

Discussion

Analyzing the situation regarding the quantitative changes of glutathione reductase and glutathione peroxidase, it is possible to trace many new data on the line of antioxidants in ascites ovarian tumor [1, 3, 4].

It should be noted the fact that this tumor, as all tumors, has an increased metabolism. That is, you should expect a large number of unoxidized products and free radicals. Therefore, the cells will use almost all possible options for their disposal. Since it is generally known that, the reactions of glycolysis or gluconeogeogenesis for the subsequent synthesis of ATP will come to the fore. Usually the activity of proteins and enzymes to inactivate free radicals, atomic oxygen and hydrogen peroxide will be activated [1, 6].

Although in the study in the group of control pathology there was inactivation of glutathione

peroxidase, and for 4 months, the activation still increased only on 8.6 %.

In our opinion, this situation is explained by the fact of complete underoxidation of products in the Krebs cycle. That is, in the intermediate stages of decay in the first periods of life of atypical cells, they are not interested in the complete decay of molecules. Namely, the cell does not use the full potential of macroergs. Or even moored, may be in the absence of enzymes for complete disengagement. It is possible that the cells are dominated by anaerobic glycolysis [7].

At a later stage in the existence of atypia, however, molecules of hydrogen peroxide appear. This is evidenced by activation of glutathione peroxidase, although the activity of glutathione reductase is much higher in the group of control pathology.

In the group without pharmacological correction, initially there is decrease in the activity of glutathione reductase. This is also one example of the fact that cells in a tumor do not completely break down metabolites. Usually for this substrate in the form of glutathione requires less. The reasons for this phenomenon are probably the same factors that we gave in the first version [6].

After 4 months of the study, glutathione reductase activity also began to increase. This value is due to the same factors as in the first case. Namely, the substrate in the form of glutathione for utilization, such as atomic oxygen, becomes larger compared to the early stages of the functioning of atypia [2].

Immediately with the use of gammoglobulins, the patterns of growth of glutathione reductase and glutathione peroxidase began to differ sharply.

At the 2nd month of application of protein structures, there was increase in the activation of glutathione peroxidase. That is, hydrogen peroxide molecules began to accumulate in the cells. It has been shown that metabolites are part of energy metabolism practically break down into their final metabolites [1, 8].

At the 4 month, the concentration of hydrogen peroxide in the cells began to decline. This is probably due to the fact that the cell develops enzymatic systems that do not synthesize hydrogen peroxide, such as atomic oxygen or free radicals. In our opinion, an atypical cell with large energy reserves and action on the non-damaging factor in the form of gamma globulin is considered the most detrimental factor in the action of hydrogen peroxide molecules on it [9].

Diametrically different situation arose with glutathione reductase. Namely, initially under the action of gammaglobulins, there is decrease in the concentration of this enzyme. That is, glutathione has smaller role in redox reactions.

Already in the 4th month of administration of gammaglobulins there is increase in enzyme activity. That is, glutathione becomes one of the central coenzymes in bioreduction reactions in atypical cells [8].

After the introduction of gammaglobulins, there are differences in redox reactions in atypical cells. Moreover, these changes differ significantly in the 2nd and 4th months.

That is, probably gammaglobulins, have a cytotoxic effect on atypical ovarian cells, which are expressed not only in the action of redox reactions, but also on energy metabolism in cells. These moments significantly affect the increase in the concentration of the final products of metabolism [4, 8].

As a way out, we see a positive therapeutic effect of gammaglobulins, as the facts we mentioned above affect the atypical cell and encourage it to survive by transferring the production of macroergs in easier ways. This in turn will give a low energy potential of the cell, which will lead to an acidotic variant of cell death.

Conclusions

1. The glutathione system in the form of glutathione reductase and glutathione peroxidase is inactivated after 2 months, but at 4 months of study the activity of these enzymatic systems increases.

2. Treatment with gammaglobulins gives a certain therapeutic effect due to a change in the activity of the studied enzymes involved in redox intracellular reactions.

3. It was established that for 2 months activation of glutathione peroxidase with parallel inhibition of glutathione reductase. However, for 4 months the activity changed in the opposite direction.

Acknowledgments

The authors declare that there are no conflicts of interest.

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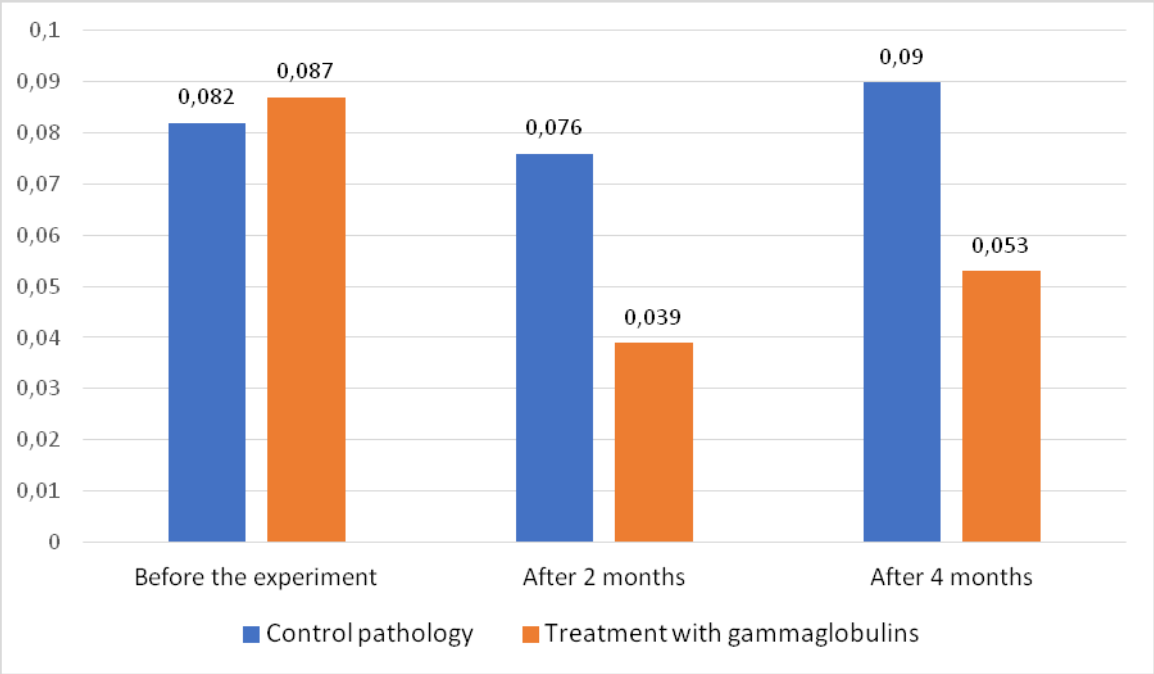

Figure 1. Change the glutathione reductase level in experiment, U/l

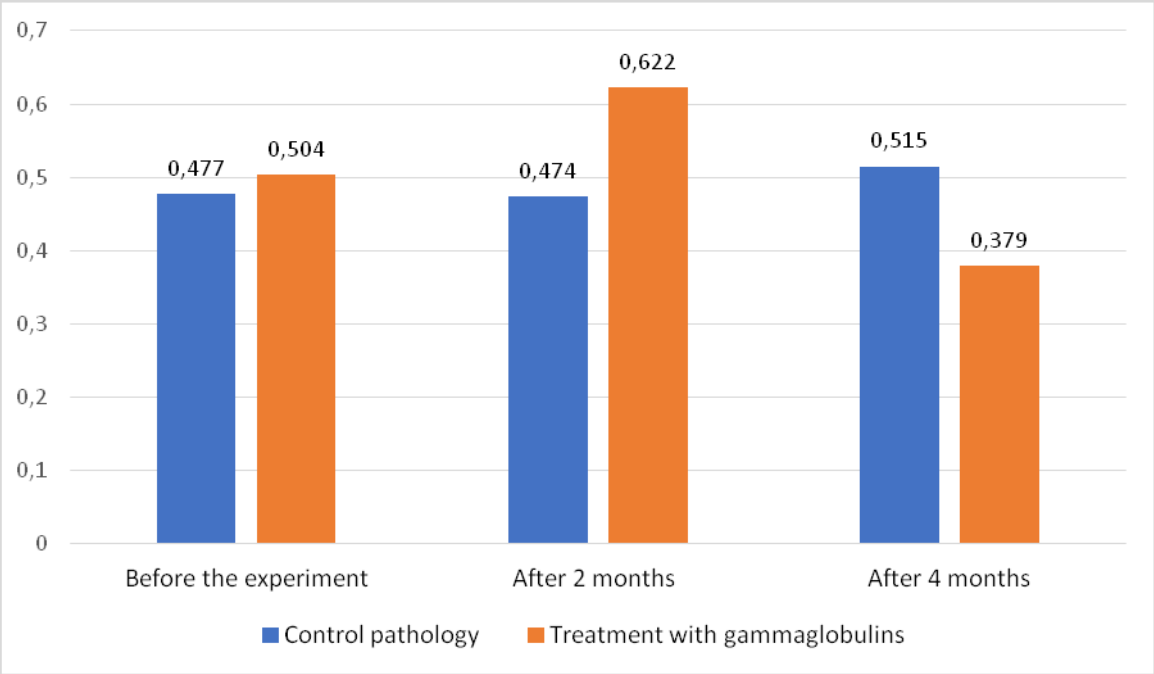

Figure 2. Change the glutathione peroxidase level in experiment, U/l

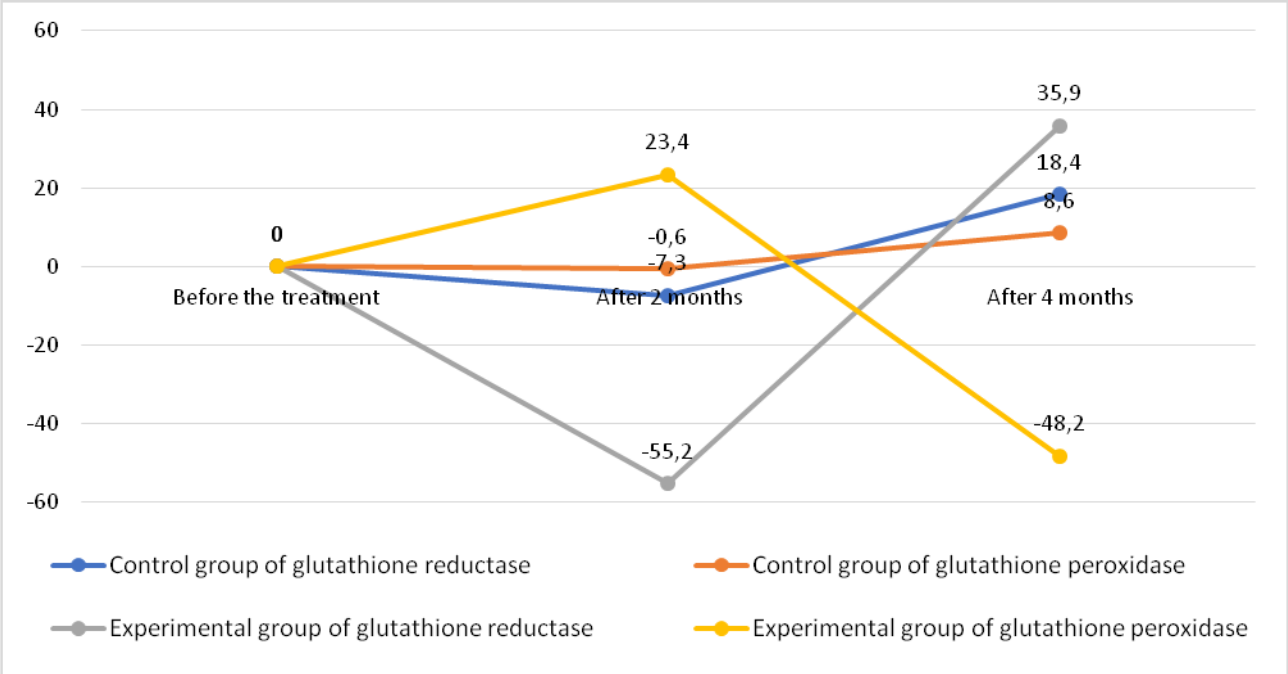

Figure 3. Comparison of glutathione reductase and glutathione peroxidase activity, %