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LONG-TERM RESULTS OF THE ENZYME GLUTATION SYSTEM ACTIVITY IN EXPERIMENTAL OVARIAN CANCER

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Abstract

The article presents the experimental results of the investigation the glutathione system activity (glutathione peroxidase and glutathione reductase) in rats simulated ovarian ascites tumor. In the experimental carcinogenesis of the ovaries, the activity of glutathione reductase and glutathione peroxidase was inhibited at all intervals of the study during long-term observation. After the presence of carcinogenesis in the body, the number of reactions that do not lead to complete utilization of metabolites. Glutathione reductase activity increases between 4 and 6 months. And the activation of glutathione peroxidase for the entire period only decreases. The experiment showed that gammaglobulin with long-term treatment can increase the activity of the glutathione system.

Keywords: ascitic ovarian tumor; glutathione system; reductase; peroxidase; cancerogenesis.

Introduction. Authors who study the subject of carcinogenesis in most cases the investigation is conducted in a clinical setting. Such given conditions cannot provide answers

to the stage of tumor change and more accurate pathophysiological variants of carcinogenesis. The second problem of this method is practically descriptive passage of all pathogenetic links against the background of concomitant pathology. And research with chemotherapeutic drugs requires significant clinical effort to select related groups [5, 6].

Numerous authors in medical and biological practice have paid much attention to oxidative stress in recent years. There is a group of scientists who single out this key link. However, there is an opinion that such a situation is possible and may even initiate carcinogenesis. Glutathione plays a significant role in this system. Since glutathione is present throughout eukaryotes, it may also play an important role in inhibiting the development of atypia [1, 3, 4].

The aim of work to monitor changes in glutathione reductase and glutathione peroxidase activity after 4 and 6 months of ovarian cancer modeling in rats.

Materials and 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. At the beginning of the experiments, after modeling of ovarian carcinogenesis with histological control, we obtained different indicators of enzyme activity in the body. Thus, in groups the indicator ranged from 0.006 U/l. In general, this is 7.3 % of the activity in the control group (Fig. 1).

Changes in glutathione reductase activity in the control group can be observed in the remote results. Namely, after 4 months of studying, the activity increased by 0.008 U/l. In general, in the control group during the study, the activity of glutathione reductase increased by 0.007 U/l (8.5 %).

In the group without correction, the activity of the enzymatic system at the beginning of the experiment was lower by 0.001 U/l, which is 1.2% of the control group.

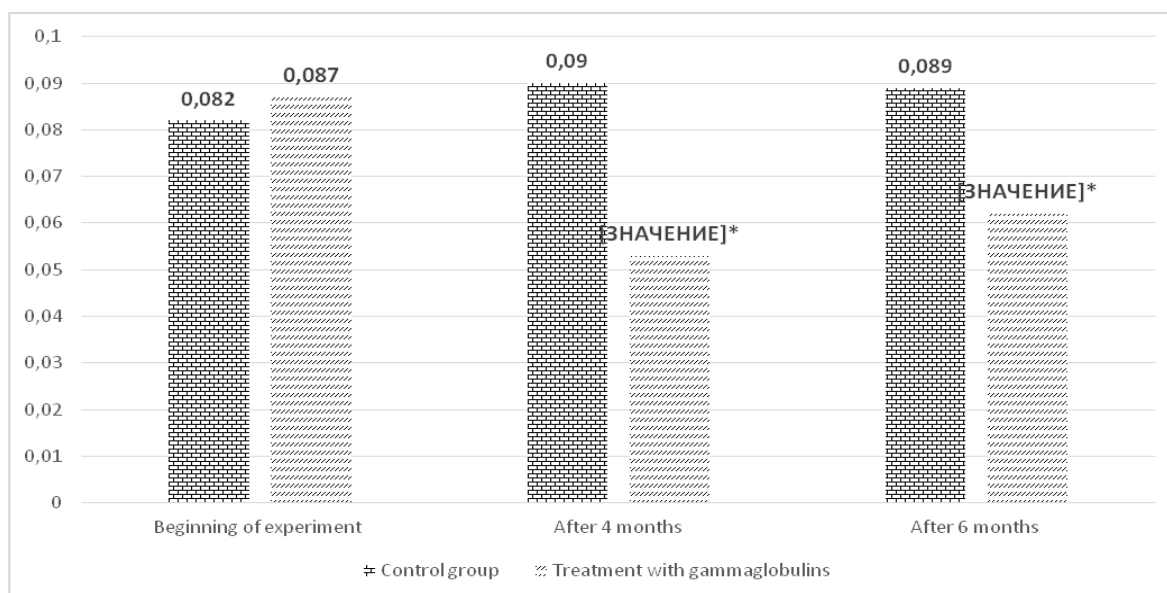


Fig. 1. The changes of glutathione reductase activity, U/l

Note. * - $p < 0,05$ compared with the control group.

After 4 months, the study showed a significant decrease in activity by 0.03 U/l, which is equivalent to 37.0 %. At 6 months of studying, the value in the group begins to increase by 0.013 U/l (16.0 % of initial dates). In general, in the group with correction we established the decrease of glutathione reductase activity by 0.017 U/l (20.98 %).

Concluding the change in the glutathione reductase activity, it should be noted that the long-term results of the activity of the enzymatic system is not as pronounced as in the simulation of the pathological condition. Lower rates of inhibition of the enzymatic system in the long term, was observed in the group without treatment by gammaglobulin. Thus, the difference from the initial value decreased by 20.98%.

Although, at the same time in the gamma globulin group, the inhibitory activity was more pronounced at 4 months. Although the activity increased at 6 months, the overall value of inhibition was 28.7 %.

Before the beginning of the study, different indicators of glutathione peroxidase activity were observed in all groups. The average value is 0.499 I /l, which is 0.022 U/l more than the control (4.6 %). In the control group after 4 months, the enzymatic system increased by 0.038 U/l (7.97 % from the beginning of the experiment). On the long-term result, the indicator decreased by 0.055 U/l (11.5 %) (Fig. 2).

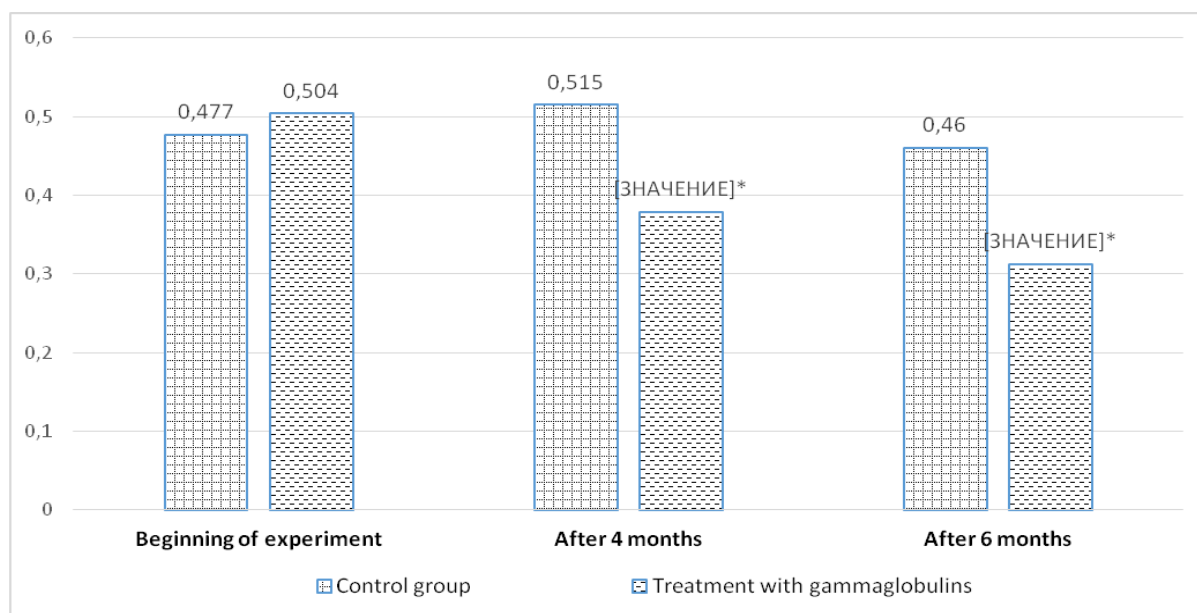


Fig. 2. The changes of glutathione peroxidase activity, U/l

Note. * - $p < 0,05$ compared with the control group.

In general, in the control group on the studied terms, the activity of glutathione peroxidase decreased by 0.017 U/l (3.6 %). The initial activity of glutathione peroxidase in the group with gammaglobulin treatment were provided, the initial level was higher than the control group by 0.027 U/l which is expressed in 5.7%. In the same situation, the activity of the enzyme also begins to decline. Thus, after 4 months of treatment, the enzymatic activity decreased by 0.125 U/l (24.8 %). Subsequently, after 2 months, the activity decreased by 0.067 U/l (13.3 %). Thus, at long intervals, the activity of glutathione peroxidase decreased significantly in the group with gammaglobulins treatment. In absolute terms, this is a decrease of 0.192 U/l (38.1 %).

From Fig. 3 clearly shows an increase in the activity of the enzymatic system. Thus, glutathione reductase and glutathione peroxidase increase their activity in experimental animals. Although the activity of glutathione reductase is higher at all stages. The values are 9.8 % and 8.5 %, respectively, compared to 7.97 % and 3.6 %. In groups, in the presence of carcinogenesis, the activity of the enzymatic system is inactivated at all stages.

That is, in a detailed analysis of the group with the experimental ovarian tumor glutathione reductase is 37% inactivated at 4 months of study. At the same time, the level of inactivation of glutathione peroxidase reaches 25.8 %. This is 11.2 % less than glutathione reductase.

After 6 months of studying is as follows: glutathione reductase increases its activity, although glutathione reductase further reduces its activity. Thus, the first enzyme reduced its activity, compared with initial dates, by 20.98 %. The second enzyme in total reduced its activity by 35.5 %. That is, compared with the original data, peroxidase reduced its activity by 14.52 %.

In the gammaglobulin correction group, the situation was identical, but with different relative values. At the 4th month of the study, glutathione reductase reduced activity by 39.1 %, while glutathione reductase by 24.8 %.

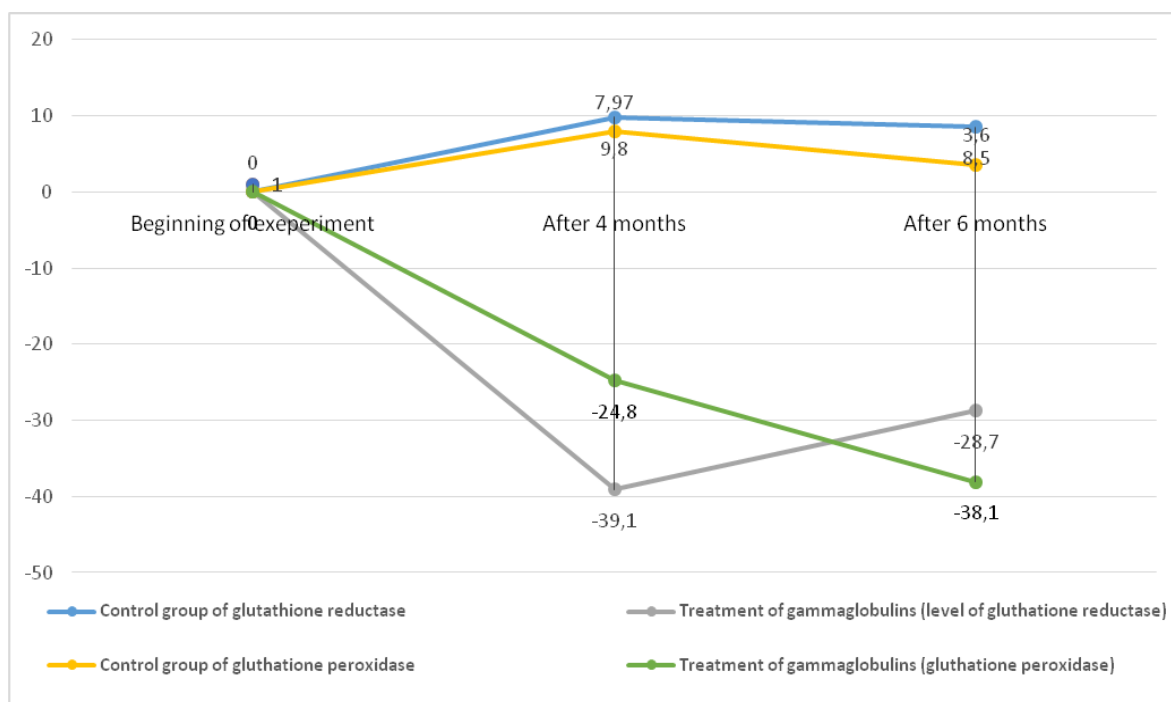


Fig. 3. Changes in glutathione reductase and glutathione peroxidase activity, %.

At 6 months, the first enzyme increased its activity to (-28.7%) compared to initial, but inactivation of glutathione peroxidase began to increase to 38.1 % of the deficit from baseline. The analysis shows decrease in glutathione peroxidase activation compared to glutathione reductase at the 6th month of the study in the amount of 9.4 %.

Based on the fact that with long-term observation, the activity of the enzymatic system increases. That is, cells with normal functioning and the predominance of anabolism and complete utilization of substrates, there is a need for utilization of free radicals. Therefore, there is a situation with increased glutathione reductase and glutathione peroxidase. The cell is actually using its full potential. In groups when modeling ovarian carcinogenesis, there is a situation with the inactivation of enzymatic systems for the utilization of free radicals. We have previously described this situation in the early stages of the study. A similar situation occurs in the long-term studying.

Glutathione reductase is more inactivated at 4 months compared to glutathione peroxidase. Biochemically, this phenomenon is explained by the fact that in an atypical cell in the first place is glutathione in redox reactions. And free radicals in the cell become less. An atypical cell by its nature does not oxidize macroergs to the final metabolites. Therefore, there is no increase in such an enzymatic system [7, 8, 9].

At the 6th month, we observed in the graphs the moment when glutathione entered into redox reactions more often than at the 4th month. Therefore, the activity of glutathione

reductase increased, although in general the activity remained lower compared to initial level. But glutathione reductase activity continued to increase what can be explained as a conclusion about the atypicality of metabolism in carcinogenic cells and in the body. In our opinion, in such a situation, first of all, a large number of deoxidized products will grow in the cell, which in fact have an acidic reaction. This only confirms the opinion of many authors that tumors contain a large number of dead cells.

In this case, for gammaglobulin correction, which is essentially responsible for enhancing the utilization of underoxidized products of atypical cells. From the results of the experimental study, we saw a difference between the two options. Namely, we improved the situation compared to the group that had atypical cells. However, even after 6 months of treatment, in experimental conditions, we did not achieve the result of the control group and the starting point [8].

However, the enzymatic activity in the body was improved at 6 months. However, the therapy slightly reduced the atypia of metabolism in the cell. The task of restoring reactions aimed at complete cleavage of metabolites was not achieved, due to the fact that we did not act on the synthesis of enzymes involved [9].

Conclusions:

1. In the experimental carcinogenesis of the ovaries, the activity of glutathione reductase and glutathione peroxidase was inhibited at all intervals of the study during long-term observation. After the presence of carcinogenesis in the body, the number of reactions that do not lead to complete utilization of metabolites.

2. Glutathione reductase activity increases between 4 and 6 months. And the activation of glutathione peroxidase for the entire period only decreases.

3. The experiment showed that gammaglobulin with long-term treatment can increase the activity of the glutathione system.

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