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# Antioxidant Therapy as a Protector and Corrector of Haematological Toxicity of Systemic Therapy for Breast Cancer

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**Abstract**

Side effects of anticancer drugs seriously limit the achievement of the maximum therapeutic effect of the most cytostatics. Pathophysiological basis of side effects is the ability of cytotoxic agents to intensify the free-radical processes and the con-

sequent lipid peroxidation (LPO) in the cell membranes of various organs. In our study we observed the efficacy of antioxidant therapy in the prevention of the development of leukopenia. It was discovered that administration of antioxidant therapy during course of chemotherapy for breast cancer leads to significantly lower incidence of leukopenia (78.5% of cases in control group versus 43.8% in study group with study drug), and lower incidence of deep leukopenia. Also in was found that patients with a low level of SH-groups (less than 1 mmol / g protein) in parallel activation of oxidative stress are in the risk group for the occurrence of neutropenia in the background of breast cancer chemotherapy and require dynamic observation with individual selection of donators thiols and drugs with proven antioxidant effect. Reduction in the activity of antioxidant enzymes of glutathione level and the displacement of the thiol-disulfide equilibrium in the direction of reducing of the content of reduced forms indicates a reliable clinical need for the personal use of drugs with glutathione-protective effect and antioxidant potential.

**Keywords**: Breast cancer, hematological toxicity, leucopenia, chemotherapy, antioxidant therapy

## Introduction

The effectiveness of the complex therapy of breast cancer depends on the accuracy of performance standards and compliance with therapeutic measures time intervals between steps of special treatment [9]. An important condition for strict implementation of the standard of special treatment of malignant disease is a good tolerability of therapy. Side effects of anticancer drugs seriously limit the achievement of the maximum therapeutic effect of the most cytostatics. It is known that the toxic effects of anticancer drugs on hematopoiesis is the most common side effects of the treatment of patients with malignant neoplasms [4].
Pathophysiological basis of side effects is the ability of cytotoxic agents to intensify the free-radical processes and the consequent lipid peroxidation (LPO) in the cell membranes of various organs. Against the background of growth of the tumor in the body there is a shift of prooxidant-oxidant balance, which develops antioxidant enzyme deficiency of first line of defense - SOD, glutathione peroxidase and catalase [7]. Such a state is explained by developing hypoglycemic tumor pressure on normal tissue metabolism, which in turn leads to the tissues mobilization of as energy resources from fatty acids. This fact, plus the growing against the background of growth of tumor tissue hypoxia, lead to a marked enhancement of lipid peroxidation in the body [8]. Moreover, tumor cells themselves are characterized with generally low LPO rate [3]. When the antitumor treatment is conducted, the antioxidant enzyme deficiency increases, there is depletion of non-enzymatic and enzymatic mechanisms oxidation protection units, resulting in a reduction of organism resistance and damage to vital organs and systems [6]. In this connection it is important to study the possibility of correction

of violations occurring in cancer patients with drugs with antioxidant action type [2, 1].

The objectives of this study were: 1) to study the efficacy of antioxidant therapy in the prevention of the development of leukopenia, as well as the possibility of improving treatment outcomes in their use; 2) to investigate the influence of metabolic disorders of thiol-disulfide system to the risk of haematological disorders (development of agranulocytosis) after chemotherapy in breast cancer; 3) to justify the use of antioxidant therapy for the prevention and treatment of hematological toxicity of chemotherapy.

Materials and methods. The study of hematological parameters was evaluated in two groups of patients – control group and the study group with study drug. All patients were women with histologically and immunohistochemistry verified breast cancer. Both groups of patients received chemotherapy for breast cancer with taxanes+anthracyclines. Cancer drug administration mode - 1 every 21 days with a weekly control of blood parameters. Both groups of patients received standard premedication on the day of administration of chemotherapy. In the period between courses of study group patients received thiotriazoline antioxidant in a dose of 200 mg orally 2 times a day. In the case of leukopenia both groups received the standard treatment for indications (colony stimulating drugs, hormones, antibiotic therapy for febrile neutropenia prophylaxis), in addition, the patients received treatment group injecting thiotriazoline 2.5% 4.0 ml / 1 time per day to normalize blood parameters. We studied the incidence of leucopenia during treatment, her relationship with co-morbidities, changes in other indices of blood tests, diagnosis. Monitoring of biochemical blood analysis was conducted 1 time in 3 weeks before the next course of chemotherapy, blood count - on a weekly basis. To study the parameters of oxidative stress in patients during the chemotherapy blood samples of patients were taken one day before the chemotherapy course and the next day after the injection of cytostatics. Markers of protein oxidative modification, system of nitrogen oxide, thiol system, apoptotic markers were studied [5].

The number of patients in the control group were 110 people, in a group of study drug - 80 people.

Laboratory (hematology) indicators were studied with analyzer BC-1800 Auto Hematology Analyzer. Biochemical parameters were studied with biochemical and enzyme immunoassay automated analyzer Biochem Analette. For the determination of oxidative stress markers and state of the antioxidant system in patients one day before a course of chemotherapy and one day after administration of cytotoxic drugs blood sampling was carried out. By centrifugation, the blood serum is released. Defined markers of oxidative modification of proteins, a system of nitric oxidehe thiol-disulfide balance and apoptosis markers according to standard procedures have been studied.

Processing studies were carried out using conventional methods of mathematical statistics: for quantitative traits - parametric methods, and for the quality - nonparametric.

Results and its discussion. In the control group of patients during 1 course of treatment leukopenia was recorded in 78.5% of cases, in the course of 2 - in 79.5% of

cases. In the study group of patients leukopenia was found in 43.8% of cases, in the course of 2 - in 36.2% of cases. The differences between the groups of patients are reliable.

Consider the dynamic change of blood parameters. When monitoring the blood parameters at 1 week of 1 cycle of the chemotherapy the incidence of leukopenia in the control group was as follows: 1 degree of leukopenia - 16.4% (18 cases), leukopenia 2 degree - 24.5% (27 patients), leukopenia 3 degree - 22.7% (25 cases), leukopenia 4 degree - 7.3% (8 cases). In the study group next picture of hemogram was observed: leukopenia, 1 degree - 6.3% (5 cases), leukopenia 2 degree - 16.3% (13 patients), leukopenia 3 degree - 1.3% (1 case), leukopenia 4 degree - 1.3% (1 case). In the group of patients receiving thiotriazoline, the incidence of leucopenia was significantly lower ($p < 0.001$).

At 2 week of 1 cycle of special treatment in the control group of patients the following pattern parameters of blood were observed: leukopenia, 1 degree - 18.2%, anemia 1 degree - 42.7%; leukopenia 2 degree - 16.4% (18 cases), anemia 2 degree - 5.5%; leukopenia 3 degree - 4 cases (3.6%), leukopenia 4 degree was not met. In the study group significantly fewer indicators abnormalities were observed ($p < 0.05$): leukopenia, 1 degree - 11.3%, anemia 1 degree - 8 cases (10%); leukopenia 2 degree - 6 patients (11.2%), anemia 2 degree was not met; leukopenia 3 degree - has not been observed, 2 cases of leukopenia 4 degree.

At 1 week, 2 cycles of chemotherapy were recorded the following changes in blood count. The incidence of leukopenia was significantly higher in the control group - 70.4% in comparison with the test group (28.7%). The differences were significant ($p < 0.001$). A similar pattern was observed in the hemogram of 2 week of 2 cycle of chemotherapy. In the control group of patients the frequency of abnormalities was: leukopenia, 1 degree - 19.4% (16 cases); leukopenia 2 degree - 14.8% (16 cases); leukopenia 3 degree - 4.6%. In the study group of patients the frequency of registration of hematological complications was significantly lower: leukopenia, 1 degree. - 4 cases (5%); leukopenia 2 degree. – 3 cases (3.8%); leukopenia 3 degree was not recorded.

Prooxidant-oxidative balance indicators in the plasma of patients were studied (tab. 1).

| Group | SH-groups, mmol/g | Spontaneous oxidative protein modification | | Stimulated oxidative protein modification | |
|---|---|---|---|---|---|
| | | Adelhyd-fenihydrazon, y.e./g | Ketone-fenihydrazon, y.e./g | Adelhyd-fenihydrazon, y.e./g | Ketone-fenihydrazon, y.e./g |
| Control | 0.44 | 3.63 | 2.51 | 5.36 | 3.65 |
| Study | 0.71 | 4.44 | 3.20 | 7.42 | 3.51 |

Table 1. Examples of indicators of prooxidant-oxidative balance in patients of the control and study groups after first cycle of chemotherapy

To study the effect of prooxidant-oxidative balance in the risk of agranulocytosis, we conducted a logistic regression analysis with the calculation of the regression coefficients, Wald statistics, $\chi 2$ and odds ratio with 95% confidence intervals (95% CI). After the appropriate calculations it was proved that the thiols levels less than 1 mmol/g protein in conditions of intensification of free radical oxidation (level of stimulation AFG over 6 y.e./g protein (relative risk for this cohort of patients was 5.83 95% CI (3.47; 9.61)) significantly increased by almost 6 times the probability of "myelosuppression as a complication of chemotherapy," according to the resulting odds ratios compared with patients in whom the above-mentioned indicators were within normal limits, $p < 0.01$.

Analysis of the data showed that in patients with low levels of recovered (the SH) -groups thiols, insufficient activity of GTP in a nitrosating stress (disturbance of NO metabolism due to exposure to reactive oxygen species) frequency of post-chemotherapy patients with leukocyte levels below $4 \times 109 / l$ ($\chi^2 = 24,6$ $p < 0.01$) was recorded significantly frequently.

Nitrosating stress leads to a deficiency of reduced glutathione, which contributes to elevation of cytotoxicity of NO derivatives, in turn, the formation of deficit of reduced forms of glutathione under oxidation processes intensification in patiens with breast cancer reduces bioavailability of nitric oxide, determinates synthesis of nitrogen dioxide, which nitrosylate thiols, additionally uses cell glutathione reserves and aggravates thiol-disulfide balance after chemotherapy.

Conclusions: 1) In patients of the study group receiving thiotriazoline in preventive and treatment regimens, the incidence of leucopenia was significantly lower ($p < 0.001$) than in the control group of patients. Thus there is a significantly lower incidence of deep leukopenia (2 and higher degrees) than the control group.

2) Patients with a low level of SH-groups (less than 1 mmol / g protein) in parallel activation of oxidative stress are in the risk group for the occurrence of neutropenia in the background of breast cancer chemotherapy and require dynamic observation with individual selection of donators thiols and drugs with proven antioxidant effect.

3) Reduction in the activity of antioxidant enzymes of glutathione level and the displacement of the thiol-disulfide equilibrium in the direction of reducing of the content of reduced forms indicates a reliable clinical need for the personal use of drugs with glutathione-protective effect and antioxidant potential, which is the Thiotriazoline, clinical testing of which was carried out in our work .

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