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CORRECTION OF THROMBOCYTOPOIESIS IN RATS WITH WALKER-256 CARCINOMA USING AN ANTIOXIDANT SUPPLEMENT IN THE SETTING OF CYTOSTATICS

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ABSTRACT — In this study, we've evaluated the myelotoxic effect of treating Walker-256 carcinoma with cytostatics, followed by correction of thrombocytopenia with liposomal and liposome-free mexidol. The study included 60 rats, which, depending on the type of treatment, were divided into 5 groups. We recorded the greatest increase in the number of platelets on the background of liposomal mexidol both on the 3rd and 7th day after the start of chemotherapy. At the end of the monitoring, the myeloprotective effect was 30% higher in the liposomal mexidol compared to its non-liposomal form. Conclusion: Activation of thrombocytopoiesis on the background of cytostatic therapy helps to reduce the complications related to the use of chemotherapy.

KEYWORDS — Walker-256 carcinoma, platelets, megakaryocytes, thrombocytopenia, doxorubicin, mexidol.

INTRODUCTION

Breast cancer occupies the leading place among malignant neoplasms in women [1, 2, 3, 4]. At the same time, cytostatics are widely used in the treatment of breast cancer [5]. In the setting of cytostatic therapy, side effects often develop: anemia, thrombocytopenia, neutropenia, bleeding, etc. [6]. Myelosuppression is the main reason for limiting the dose of cytostatics in the treatment of breast cancer.

It's also known that antitumor agents cause a strong oxidative stress in the body in combination with the depletion of antioxidant protection, which increases the inhibition of the myelopoietic germ [7]. Therefore, the search for effective methods for regulation of thrombocytopenia, which is a common complication in cancer patients receiving cytostatic therapy, poses one of the urgent challenges to modern medicine.

Aim: to estimate the effectiveness of antioxidant therapy in the correction of thrombocytopoiesis in rats with Walker-256 carcinoma on the background of cytostatic therapy.

METHODS

The experimental work was performed on 60 female Wistar rats weighting 160–270 g. All procedures with animals were performed according to the guidelines for the care and use of laboratory animals. The tumor process was modeled by introducing a suspension of Walker-256 (W-256) carcinoma cells under the skin of the rat tail, followed by histological verification of the neoplasm. For cytostatic therapy, we used doxorubicin hydrochloride ("Pharmachemie", the Netherlands) — 0.04% solution, which was diluted with isotonic sodium chloride solution. To correct thrombocytopoiesis in rats with Walker-256 carcinoma, we used Mexidol (Emoxypine); Pharmasoft Pharmaceuticals, Russia) both in its free and liposomal form. In both cases, we used Mexidol at a dose of 50 mg/kg intravenously daily from the beginning of the use of cytostatics for 7 days.

Depending on the chosen treatment, all animals were divided into 4 groups:

group 1 (n=12): intact rats — these animals were not manipulated;

group 2 (n=12): rats with transplanted Walker-256 carcinoma that do not receive medication;

group 3 (n=12): rats with Walker-256 carcinoma receiving doxorubicin hydrochloride 4 mg/kg once intravenously on the 11th day after dissemination of tumor cells;

group 4 (n=12): rats with Walker-256 carcinoma, receiving doxorubicin hydrochloride 4 mg/kg once intravenously on the 11th day after dissemination of tumor cells and liposome-free mexidol at a dose of 50 mg/kg intravenously daily from the beginning of the use of cytostatics;

group 5 (n=12): rats with Walker-256 carcinoma, receiving doxorubicin hydrochloride 4 mg/kg once

intravenously on the 11th day after dissemination of tumor cells and liposomal mexidol at a dose of 50 mg/kg intravenously daily from the beginning of the use of cytostatics.

Liposomes were obtained by phase reversal from lecithin and cholesterol. The drug was encapsulated by the method of passive loading. To create liposomes, a Heidolph rotary evaporator (Germany) and a LISEC extruder (Canada) were used.

On the 3rd and 7th day after the administration of cytostatics, 6 animals from each group were removed from the experiment under general anesthesia with sodium thiopental (50 mg/kg). We counted the number of platelets in the blood of experimental animals in the Goryaev chamber. Using light microscopy, we examined bone marrow smears from the rat femur and counted the number of megakaryocytes.

Statistical processing was carried out with the calculation of arithmetic mean values (M) and their errors (m). The reliability of differences in the groups was calculated using the Mann-Whitney test. The differences were considered significant at $p<0.05$.

RESULTS

Administration of doxorubicin to rats with Walker-256 carcinoma led to the development of thrombocytopenia. The dynamics of changes in the number of platelets in the observed groups of rats is shown in Fig. 1. Thus, on Day 3, the most severe thrombocytopenia was observed in group 3: the level of platelets decreased by 37% ($p<0.01$) in relation to intact rats. Besides, in this group of animals, the inhibition of the myeloid growth was expressed almost twice as much by the 7th day of the experiment. In rats that received Mexidol in a liposomal form during a week, a dynamic increase in the number of platelets was observed: by 40.3% in relation to the 3rd group of animals; by 17.7% in relation to the 4th group of animals (Fig.1).

The dynamics of changes in the number of megakaryocytes in the rat bone marrow in all groups is shown on the Fig. 2.

In the bone marrow on the 3rd day after chemotherapy, only in animals of the 3rd group, the number of megakaryocytes significantly decreased by 80% ($p<0.05$). By day 7, the number of megakaryocytes in group 3 exceeded the baseline level in intact rats by 3 times ($p<0.05$, Fig. 2). In the bone marrow on the 3rd day after the administration of cytostatics in the 4th group of rats, the content of megakaryocytes did not significantly differ from the indicators for this criterion among intact rats. On the 7th day, the number of megakaryocytes in the 5th group of animals was significantly higher by 58.3% compared to the initial values; by 56.9% in relation to the second group of animals; by 36.3% in relation to the 4th group ($p<0.05$, Fig. 2).

DISCUSSION

The main toxic effects of doxorubicin are anemia, leukopenia, and thrombocytopenia. Moreover, the hematological toxicity of this drug sometimes reaches the 4th degree [8]. Our study showed that the exposure to doxorubicin in rats with Walker-256 carcinoma led to the development of thrombocytopenia with a tendency to reduce the number of megakaryocytes in the bone marrow by the 3rd day after the initiation of doxorubicin therapy. However, thrombocytopenia can be caused not only by the inhibition of platelet growth of hematopoiesis, but also by the direct damaging effect of cytostatics on platelets. It is known that reactive oxygen species cause platelet apoptosis via the Extracellular Receptor Kinase signaling pathway (ERK), which is characteristic of some cytostatics [7]. Such a phenomenon as: an increase in the number of megakaryocytes in the bone marrow in groups 3, 4 and 5 of rats by the 7th day after the after the initiation of doxorubicin therapy, we take as a compensatory

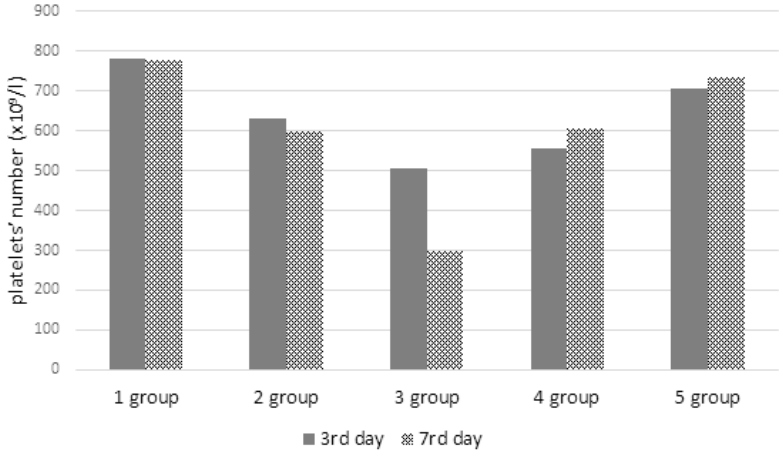

Fig. 1. Dynamics of changes in the number of platelets in the observed groups of rats

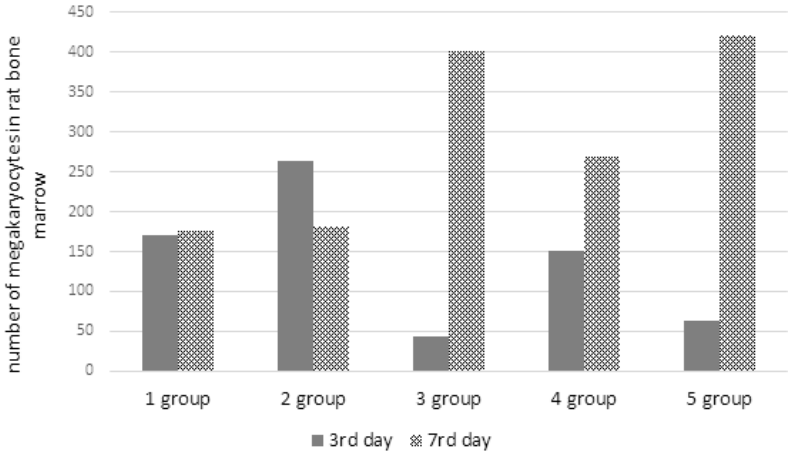

Fig. 2. Changes in megakaryocyte volume in the rat bone marrow in all groups

reaction in response to the developed and persistent thrombocytopenia. However, the liposomal mexidol had a greater myeloprotective effect compared to its free form with advantage of 30%, which confirms higher effectiveness of the drug.

Thus, our study showed that the preservation of the initial number of platelets in the peripheral blood on the 7th day after the cytostatic treatment supplemented with the use of liposomal mexidol resulted in a significant increase in the number of megakaryocytes in the rat bone marrow compared to the control group, which may indicate the activation of thrombocytopoiesis.

CONCLUSION

Liposomal mexidol (50 mg/kg), in contrast to its free form, prevents the development of thrombocytopenia induced by cytostatic therapy in rats with Walker-256 carcinoma. Activation of thrombocytopoiesis during cytostatic therapy helps to reduce the complications, related to the use of chemotherapy.

REFERENCES

1. **Rom J, Bechstein S, Domschke C, Golatta M, Mayer C, Heil J, Thum J, Smetanay K, Windemuth-Kieselbach C, Wallwiener M.** Efficacy and toxicity profile of pegylated liposomal doxorubicin (Caelyx) in patients with advanced breast cancer. Anti-cancer drugs. 2014; 25(2): 219–224. DOI: 10.1097/CAD.0000000000000037
2. **Vykhristyuk Yu.V., Roitberg G.E., Dorosh J.V., Karaseva N.V., Akobova R.A.** Preventive measures against development of breast cancer. South Russian Journal of Cancer. 2021;2(1):50–56. https://doi.org/10.37748/2686-9039-2021-2-1-6
3. **Troshenkov E.A., Kostin A.A.** Problems of diagnosis and treatment of primary operable breast cancer. Research and Practical Medicine Journal. 2015; 2(2):35–43. https://doi.org/10.17709/2409-2231-2015-2-2-35-43
4. **Roytberg G.E., Kondratova N.V.** Screening and prophylaxis of breast cancer in primary care. Bulletin of Roszdravnadzor. 2018 (1). P. 57–61.
5. **Inchina V., Izbastyyeva M., Tarasova T., Ulanova T., Avanesov A., Khaydar D.** Antitumor efficacy of liposomal doxorubicin hydrochloride in combination with tamoxifen. Experimental study. Archiv Euromedica. 2020; 10(3): 22–24. DOI: 10.35630/2199-885X/2020/10/3.4
6. **Avisar N, Adar L, Bock J, Muller U, Shen D, Barash S, Pukac L.** First-in-human, phase I/IIa dose-escalation and safety study of balugrastim in breast cancer patients receiving myelosuppressive chemotherapy. Cancer chemotherapy and pharmacology. 2015; 75(5): 929-939. DOI: 10.1007/s00280-015-2703-1
7. **Zhang W., Zhao L., Liu J., Du J., Wang Z., Ruan C., Dai K.** Cisplatin induces platelet apoptosis through the ERK signaling pathway. Thromb Res. 2012; 130(1): 81–91. DOI: 10.1016/j.thromres.2012.02.013.
8. **Rom J, Bechstein S, Domschke C, Golatta M, Mayer C, Heil J, Thum J, Smetanay K, Windemuth-Kieselbach C, Wallwiener M.** Efficacy and toxicity profile of pegylated liposomal doxorubicin (Caelyx) in patients with advanced breast cancer. Anti-cancer drugs. 2014; 25(2): 219–224. DOI: 10.1097/CAD.0000000000000037