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MYELOPROTECTIVE EFFECT OF COMBINED APPLICATION OF UKRAINIAN-MADE RECOMBINANT GRANULOCYTE COLONY STIMULATING FACTOR (R-GCSF) AND C2 ENTEROSORBENT IN RATS WITH MALIGNANT GUERIN CARCINOMA

The study is aimed at analyzing myeloprotective effect of new enterosorbents used separately and in combination with two recombinant granulocyte colony stimulating factors: Neupogen (Switzerland) and r-GCSF (Ukraine). The Ukrainian-made r-GCSF has been proved to be as therapeutically efficient as Neupogen (Switzerland); its combined use with C2 enterosorbent significantly increases myeloprotective effect of both GCSF versions.

Keywords: tumor, myelosupression, granulocyte colony stimulating factor, and enterosorption.

Modern chemotherapy with high doses of anticancer drugs has ensured a significant progress in the treatment of many cancers. However, high toxicity of antineoplastic drugs is one of the limiting factors for effective treatment [1–3]. Reducing the incidence of side effects of cytotoxic drugs without weakening their antitumor activity is an important task of modern oncology. Its solution will significantly improve the quality of patient life.

The use of massive chemotherapy and radiation therapy in the cancer treatment leads to the inhibition of hematopoietic function of bone marrow. An especially dangerous complication is the development of leukopenia and febrile neutropenia. Despite intensive research, the effectiveness of present-day strategies and tactics for the prevention and treatment of side effects of anticancer drugs is unfortunately limited. A pathogenetically substantiated method for the treatment of cytostatic myelosuppression is the use of chemocytokines, including granulocyte and granulocyte-macrophage colony-stimulating growth factors (GCSF and GM-CSF) [4–6].

Given the pronounced intoxication syndrome during the development of malignancy and the side effects during the treatment (inhibition of hematopoietic function of bone marrow, injury of mucosal epithelium of the digestive tract and hair follicles, suppression of reproductive function, signs of nephrotoxicity, etc.), the sorption detoxification method is considered to be an extremely promising approach to the treatment of cancer. In the literature, there are data on the efficiency of hemosorption, application treatment of wounds caused by radiotherapy and the use of enterosorption in supporting therapy of cancer patients [7—9]. However, so far, the available in-

formation about using enterosorbents for myeloprotection during the course of chemotherapy is not sufficient. There are no data on the effect of combination of enteral sorption therapy and hematopoietic growth factors for the prevention and treatment of side effects of poly-chemotherapy.

The purpose of this study is to determine the myeloprotective effect of combined use of domestic recombinant granulocyte colony-stimulating factor (r-GCSF) and C2 enterosorbent [10] in rats with Guerin malignant carcinoma. The r-GCSF was developed at the R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the NAS of Ukraine within the framework of project «Development of Technique for Producing Human Recombinant Granulocyte Colony-Stimulating Factor and Drugs Based on It,» contract no.DZ/487-2011 of 29.09.2011 financed by the government. It should be noted that a significant part of this research was the study of the effect of these agents on the dynamics of tumor growth.

MATERIALS AND RESEARCH METHODS

The experiments were conducted on Wistar male rats bred in vivarium of IEPOR of the NAS of Ukraine. Guerin carcinoma was transplanted to the animals under their skin on the back (0.4 ml of 23% suspension of tumor tissue). After tumor transplantation the rats were divided into 6 groups (7 animals in each): I – tumor control; II – L-PAM; III – L-PAM + Neupogen; IV – L-PAM + C2; V – L-PAM + C2 + Neupogen; and VI – L-PAM + r-GCSF + C2.

Within 10 days after the transplantation of Guerin carcinoma, Melphalan (L-PAM) was introduced intravenously at a dose of 5.5 mg/kg to the animals of II–VI groups. One day after administration of L-PAM, the rats started to subcutaneously administer Neupogen or r-GCSF at a dose of 300 micrograms per rat weighing 120–125 g during 4 days. The C2 enterosorbent was injected per os during 3 consecutive days before the introduction of L-PAM and 6 times daily after the administration of cytostatic agent. The dynamics of tumor growth, death of animals in the groups, and common blood values were monitored. The anti-tumor effect was assessed in 17 days after the transplantation of Guerin carcinoma. The common blood parameters were determined at Particle Counter E-210 hemoanalyzer (Erma Inc, Japan).

Morphological studies of bone marrow were carried out with the help of the Pappenheim method. The statistical data were processed using Student coefficient t.

RESULTS AND DISCUSSION

As a result, the lifetime of experimental rodents in the groups after the course of treatment has been studied. The results are showed in Table 1.

As Table shows, in 17 days after tumor transplantation, in the 1st group all 7 rats survived; in the 2nd group, 3 of 7 rodents were alive; in the 3rd group, 6 rats survived; 5 animals continued to live in the 4th group; 6 rodents remained alive in each of the 5th and the 6th group.

Also, the lymphocytic parameters of peripheral blood of laboratory rats were studied. The results showed that a single intravenous administration of L-PAM at a dose of 5.5 mg/kg led to a significant decrease in blood leukocytes (see Table 2).

The percentage of granulocytes was very low, with monocytes being absent at all. In the 3rd group (L-PAM + Neupogen), the total number of leukocytes increased 2.3 times. However, the best improvement of this indicator with almost

Table 1

Mortality Rate in I–VI Groups

Group	Number of animals died
I, tumor control	0/7
II, L-PAM (Melphalan)	4/7
III, L-PAM + Neupogen	1/7
IV, L-PAM + C2	2/7
V, L-PAM + Neupogen + C2	1/7
VI, L-PAM + r-GCSF + C2	1/7

normal of granulocyte level was reported for the 5th (LPAM + Neupogen + C2) and the 6th group (L-PAM + r-GCSF + C2). Administration of L-PAM also caused a pronounced (6.6 times) decrease in the number of thrombocytes in peripheral blood (Table 3).

Also, very low levels of this parameter were reported for the 3rd (L-PAM + Neupogen) and the 4th (L-PAM + C2) groups. However, in the 5th and 6th groups, the thrombocytes count approached the values in the control group (Table 3). It should be noted that the number of red blood cells in peripheral blood and, respectively, the hemoglobin level in the animals of all experimental groups were virtually identical.

As mentioned above, a very important component of the research was studying the effect of these agents on the dynamics of tumor growth.

The results of assessment of Guerin carcinoma growth based on changes in its volume are given in Table 4. As Table shows, in 13 days after tumor transplantation, the inhibition of tumor growth ($p < 0.05$) was reported for the «therapeutic» groups: in the 2nd, 3rd, and 6th groups, it was inhibited by 25%; in the 4th and 5th groups, it slowed down by 26%.

In 17 days after the tumor transplantation, the indicators of growth inhibition of Guerin malignant carcinoma were as follows: 42% (the 2nd group); 43% (the 3rd group); 46% (the 4th group); 47% (the 5th group); and 47% (in the 6th group).

Table 2

Lymphocytic Parameters of Peripheral Blood of Laboratory Rodents Having Guerin Carcinoma after the Course of Therapy

Group	Total number of leucocytes, $\times 10^6$/ml	% of lymphocytes	% of monocytes	% of granulocytes
I, Tumor control (n = 7)	13.9 ± 1.9	75.6 ± 1.7**	8.3 ± 1.1	16.1 ± 1.4
II, L-PAM (n = 3)	0.6 ± 0.1*	95.8 ± 5.1	0	4.2 ± 4.0*
III, L-PAM+Neupogen (n = 6)	1.4 ± 0.2* **	93.5 ± 3.2	0	6.5 ± 3.2*
IV, L-PAM+C2 (n = 5)	0.9 ± 0.05*	95.4 ± 3.4	0	4.6 ± 3.4*
V, L-PAM+Neupogen+ C2 (n = 6)	1.7 ± 0.2* **	85.1 ± 3.2	0	14.9 ± 3.2**
VI, L-PAM+r-GCSF + C2 (n = 6)	1.6 ± 0.3* **	86.8 ± 2.0	1.0 ± 0.7	12.5 ± 2.0**

Note: * — $p<0,05$ as compared with control (*group I*); ** — $p<0,05$ as compared with L-PAM (*II group*).

Table 3

Number of Erythrocytes, Thrombocytes, and Hb Level in Peripheral Blood of Laboratory Rodents Having Guerin Carcinoma after the Course of Therapy

Group	Total number of erythrocytes, $\times 10^9$/ml	Total number of thrombocytes, $\times 10^6$/ml	Hb level, g/l
I, Tumor control (n = 7)	7.73 ± 0.51	325.6 ± 35.9**	15.2 ± 0.8
II, L-PAM (n = 3)	8.70 ± 2.65	49.0 ± 11.9*	13.0 ± 3.0
III, L-PAM + Neupogen (n = 6)	10.57 ± 1.19	74.0 ± 9.5*	21.1 ± 2.5
IV, L-PAM +C2 (n = 5)	8.06 ± 0.68	60.0 ± 8.6*	15.3 ± 1.5
V, L-PAM+Neupogen + C2 (n = 6)	10.21 ± 1.00	205.6 ± 86.2**	18.8 ± 1.8
VI, L-PAM+r-GCSF + C2 (n = 6)	7.91 ± 0.81	146.0 ± 36.8* **	14.6 ± 1.7

Note: * — $p < 0,05$ as compared with control (*group I*); ** — $p < 0,05$ as compared with control L-PAM (*II group*).

The features of hematopoiesis recovery under the action of granulocyte colony stimulating factor (Neupogen and Filgrastim) combined with sorption therapy were studied in the rats carrying Guerin carcinoma with myelosuppression caused by Melphalan. The study was conducted in 17 days after the tumor transplantation.

In the bone marrow of tumor carriers (group I), granulocytic cells play dominating role in hematopoiesis. However, unlike normal hematopoiesis, in the bone marrow, the number of mature cells, band and segmented neutrophils, significantly decreases, as a result of their rapid access to the peripheral bloodstream. The majority of cells is young differentiating granulocytes (promyelocytes, myelocytes, and metamyelocytes). The number of erythroid cells decreases. Plasmatic and reticular cells are present in larger quantity. Megakaryocytes are found in normal quantity.

After the administration of Melphalan (group II), in the bone marrow of tumor carriers, hypo and aplastic changes with extremely low cellularity and fat inclusions were revealed. In the smears of bone marrow, areas with damaged cells were found. The granulocytic cells were isolated and have signs of destructive changes, vacuolation, karyorrhexis, karyolysis, and cytolysis. There were also hyperbasophilic mononuclear cells, plasma cells, plasma and reticular cell islands. The erythroid cells were numerically insignificant, without signs of destruction. No megakaryocytes were found. These data indicate that the administered dose of Melphalan (L-PAM) has a pronounce myelosuppressive effect.

Among advanced methods for the treatment of myelosupression, the most promising one is the use of colony-stimulating factors acting at the level of progenitor cells of bone marrow hematopoiesis and leading to a rapid and sustained increase in the content of leukocytes in peripheral blood. Experimental and clinical studies have showed the effectiveness of r-GCSF.

In this experiment, a registered drug Neupogen (group III) containing recombinant GCSF (Filgrastim) was used to reduce a depressing effect of Melphalan on hematopoiesis and to speed up the regeneration. Due to the action of Neupogen, in the bone marrow, the presence of hematopoiesis islands that include young granulocytic elements (myeloblasts, myelocytes, and promyelocytes) and mature granulocytes (band and segmented neutrophils and eosinophils) was established in the background of hypoplastic state. In addition, the smears had a relatively large content of lymphocytes, plasma and reticular cells, as well as a high concentration of basophils. The content of both granulocyte and erythroid cells increased in the bone marrow.

Interesting data were obtained using enterosorption to reduce the myelosuppressive effect of Melphalan (the 4th group). Without specific myelo-modulators, in the bone marrow of laboratory rodents who administered enterosorbents, an increase in the total number of cells was reported,

Table 4

Dynamics of Guerin Carcinoma Growth in I—VI Groups (volume, cm^3, *n* = 7)

Group	Кількість діб після перещеплення пухлини			
	7 days	10 days	13 days	17 days
I, Tumor control	0.9 ± 0.1	3.7 ± 0.2	7.7 ± 0.2	11.3 ± 0.2
II, L-PAM	0.9 ± 0.1	3.8 ± 0.3	5.8 ± 0.2 *	6.6 ± 0.2 *
III, L-PAM+Neupogen	0.8 ± 0.2	3.7 ± 0.2	5.8 ± 0.3 *	6.4 ± 0.3 *
IV, L-PAM +C2	1.0 ± 0.1	3.5 ± 0.2	5.7 ± 0.3 *	6.1 ± 0.2 *
V, L-PAM+Neupogen+C2	1.0 ± 0.1	3.8 ± 0.3	5.7 ± 0.3 *	6.0 ± 0.4 *
VI, L-PAM+r-GCSF+C2	1.0 ± 0.1	3.7 ± 0.4	5.8 ± 0.2 *	6.0 ± 0.3 *

Note: * — $p < 0{,}05$ as compared with tumor control (*group I*).

as opposed to individual effect of Melphalan. This largely concerned the content of plasma and reticular cells, as well lymphocytes. The presence of a small number of granulocytic and, to a greater extent, erythroid cells having various degrees of differentiation was established.

The analysis of bone marrow smears of the animals which administered Neupogen and C2 enterosorbent (Group V) to reduce the negative impact of Melphalan on hematopoiesis showed that the combined administration was more efficient than the separate use of these drugs. The bone marrow still remained hypoplastic, but the total number of myelokaryocytes increased, with granulocytic and erythroid hematopoiesis islands occurring more often.

These islands increased in size, with hematopoietic cells being located on the substrates of reticular and plasma cells. There were many lymphocytes; also, basophilic granulocytes were of frequent occurrence.

Among the specific features of the bone marrow recovery caused by combined action of r-GCSF (Filgrastim) and enterosorbent (group VI), there was a significant activation of the granulocytic cell differentiation towards the basophils, with the number of lymphocytes and plasma cells increasing. The content of neutrophilic and erythroid cells in the bone marrow was lower as compared with the case of Neupogen administration.

The r-GCSFs are known not to affect directly the erythroid hematopoiesis, however, in our experiment, the number of red blood cells in peripheral blood increased by almost 20% both for separate administration of Neupogen and for its combined use with enterosorbents. The content of erythroid cells in the bone marrow of rats of the 3rd and 5th groups was relatively high: 16.00 ± 5.36% and 25.80 ± 8.60% respectively. This effect can be explained by increased migration of stem cells to the peripheral channel, due to the action of r-GCSF, with their further settling in a more saved microenvironment.

In all cases, the increase in the number of plasma cells was accompanied by parallel increase in the number of reticular cells.

This requires further study. In the above described experiment, a dose of Melphalan was likely to be too high that caused an essential damage of hematopoietic microenvironment from which the hematopoiesis begins to recover.

CONCLUSIONS

1. The Ukrainian-made r-GCSF has been established to have as good therapeutic effect as Neupogen manufactured by Swiss corporation Roche.

2. The combination of r-GCSF and C2 enterosorbent significantly improves the myeloprotective and systemic effect as compared with each agent administered separately and prevents lethality among the laboratory rodents in the case of overdose of alkylating cytostatic L-PAM (Melphalan).

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ДОСЛІДЖЕННЯ МІЄЛОПРОТЕКТОРНОЇ ДІЇ КОМБІНОВАНОГО ЗАСТОСУВАННЯ ВІТЧИЗНЯНОГО РЕКОМБІНАНТНОГО КОЛОНІЄСТИМУЛЮЮЧОГО ФАКТОРА ГРАНУЛОЦИТІВ (Р-КСФГ) ТА ЕНТЕРОСОРБЕНТА С2 У ЩУРІВ ЗІ ЗЛОЯКІСНОЮ КАРЦИНОМОЮ ГЕРЕНА

Мета дослідження — вивчення мієлопротекторної дії новітніх ентеросорбентів окремо та в поєднанні з двома рекомбінантними препаратами гранулоцитарного колонієстимулюючого фактора: Нейпоген (Швейцарія) та р-КСФГ (Україна). Доведено, що вітчизняна версія рекомбінантного гранулоцитарного колонієстимулюючого фактору р-КСФГ за своєю експериментально-лікувальною дією не поступається офіцинальному препарату Нейпоген (Швейцарія), а комбіноване застосування р-КСФГ з ентеросорбентом С2 суттєво покращує мієлопротекторну дію обох версій КСФГ.

Ключові слова: пухлина, мієлосупресія, гранулоцитарний колонієстимулюючий фактор, ентеросорбція.

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ИССЛЕДОВАНИЕ МИЕЛОПРОТЕКТОРНОГО ДЕЙСТВИЯ КОМБИНИРОВАННОГО ИСПОЛЬЗОВАНИЯ ОТЕЧЕСТВЕННОГО РЕКОМБИНАНТНОГО КОЛОНИЕСТИМУЛИРУЮЩЕГО ФАКТОРА ГРАНУЛОЦИТОВ (Р-КСФГ) И ЭНТЕРОСОРБЕНТА С2 У КРЫС СО ЗЛОКАЧЕСТВЕННОЙ КАРЦИНОМОЙ ГЕРЕНА

Цель исследования — изучение миелопротекторного действия новых энтеросорбентов по-отдельности и в комбинации с двумя рекомбинантными препаратами гранулоцитарного колониестимулирующего фактора: Нейпоген (Швейцария) и р-КСФГ (Украина). Доказано, что отечественная версия рекомбинантного гранулоцитарного колониестимулирующего фактора р-КСФГ по своему экспериментально-лечебному действию не уступает официнальному препарату Нейпоген (Швейцария), а комбинированное применение р-КСФГ с энтеросорбентом С2 существенно улучшает миелопротекторное действие обоих версий КСФГ.

Ключевые слова: опухоль, миелосупрессия, гранулоцитарный колониестимулирующий фактор, энтеросорбция.

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