Reactions of Granulocytic Lineage of Hemopoiesis and Mechanisms of Their Development in Combined Treatment with Adriablastin and Taxotere

V. E. Gol'dberg, T. Yu. Khrichkova, V. A. Shatalova*, N. O. Popova*, E. S. Burshtein*, M. Yu. Minakova, V. V. Zhdanov, and A. M. Dygai

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 150, No. 12, pp.661-665, December, 2010
Original article submitted February 26, 2010

We studied myelotoxic effects of adriablastin and taxotere combination on granulocytic lineage cells and processes of their recovery in patients with stage III-IV breast cancer. Intensive maturation of granulocytic CFU provided regeneration of the hemopoiesis even under conditions of reduced proliferative activity of these cells, which, in turn, led to accumulation of mature and immature neutrophilic granulocytes in the bone marrow and improved reserve capacities of the neutrophil pool in the bone marrow.

Key Words: *chemotherapy; taxanes; granulocytopoiesis; precursor cells; bone marrow neutrophil reserve*

Numerous clinical studies demonstrated high efficiency and good tolerability of taxotere and adriablastin combination in the treatment of disseminated breast cancer (BC), which allowed many authors to recommend this combination as a new standard in the chemotherapy of diffuse BC [6,11].

The creation of this combination was prompted by maximum efficiency of each drug in the monotherapy regimen, the absence of cross-resistance and pharmacological interaction, different mechanisms of their effects, and different toxicity profiles, except for myelosuppressive effects [9,10]. Indeed, suppression of the bone marrow hemopoiesis, primarily granulocytic lineage, is the most common complication of taxotere and adriablastin treatment manifesting in leukopenia with predominant decrease in blood content of neutrophil granulocytes responsible for the protection against various infections and the formation of antitumor resistance [1]. Anemia and thrombocytopenia are less frequently observed [6].

Here we studied the mechanisms of suppression and recovery of granulocytopoiesis under the action of taxotere/adriablastin combination in patients with BC.

MATERIALS AND METHODS

We examined 47 patients with morphologically verified stage III-IV BC. The chemotherapy scheme included intravenous injection of 50 mg/m^2 adriablastin (Pharmacia & Upjohn) on day 1 and 75 mg/m^2 taxotere (Aventis Pharma) on day 2. The duration of each cycle was 2 days and the interval between the cycles was 3 weeks. Blood system parameters were evaluated during 3 courses of chemotherapy.

Capillary blood was analyzed before and after each course of cytostatic treatment. Prednisolone tests and sternal punctures were performed before treatment and after the first and second course of chemotherapy.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences; *Institute of Oncology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk, Russia. ***Address for correspondence:*** mmu@pharm.tsu.ru. A. M. Dygai

0007-4888/11/1506-0718

Parameters of peripheral blood (leukocytes, complete blood count) and differential cell count in the sternal puncture samples were determined using routine hematological tests [4].

Granulocytopoiesis precursor cells (CFU-G) isolated from nonadherent bone marrow karyocytes and peripheral blood on Histopaque-1077 density gradient (Sigma; cell concentration 2.0×10^5 ml) were cloned in semisolid methylcellulose medium. Intensity of differentiation of hemopoietic precursors was evaluated by the index of maturation (colonies to clusters ratio). Proliferative activity of granulocytic precursors was analyzed by the method of cell suicide using hyroxyurea [3].

Prednisolone test was used for evaluation of bone marrow neutrophil reserve [7]. Prednisolone (Gedeon Richter) was injected intravenously in a dose of 60 mg. Total leukocyte count (TLC) and the content of segmented neutrophils was determined before, and 2, 3, 4, 5, and 6 h after drug injection. The result was assessed by the release of neutrophilic granulocytes in percents of the initial level; the maximum release (percentage and absolute values) was determined.

The data were processed by methods of variation statistics using Student's *t* test [5].

The study protocol was approved by Ethical Committee of Institute of Oncology, Siberian Division of the Russian Academy of Medical Sciences.

RESULTS

In patients receiving taxotere in combination with adriablastin, TLC decreased by more than half as soon as after the first course of chemotherapy (Fig. 1, *a*). After 3-week rest, the number of leukocytes almost returned to the initial level and then decreased again after the

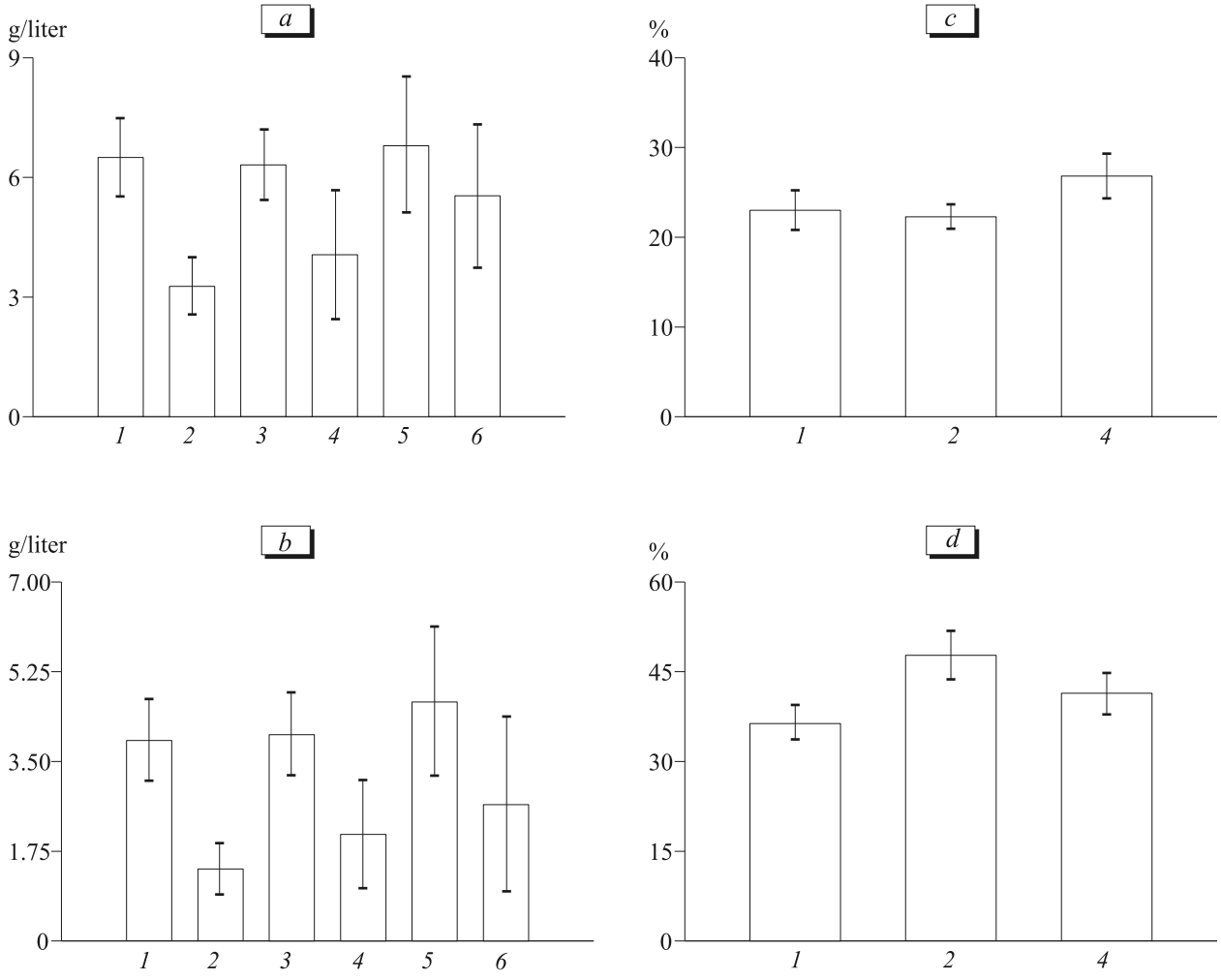

Fig. 1. Changes in TLC (*a*) and segmented neutrophil content (*b*) in the peripheral blood and immature (*c*) and mature (*d*) neutrophil granulocytes in bone marrow of patients with stage III-IV BC in the dynamics of antitumor therapy with adriablastin and taxotere. *1*) before treatment; *2*) after the first course; *3*) before the second course; *4*) after the second course; *5*) before the third course; *6*) after the third course. Here and in Fig. 2: confidence intervals at $p<0.05$.

second course of treatment, but less markedly than after the first course. Then, leukocyte count remained at a relatively high level and did not significantly differ from the values observed before cytostatic treatment (Fig. 1, *a*).

The decrease in TLC was primarily due to the decrease in the content of segmented neutrophils in the peripheral blood (Fig. 1, *b*), which was an expected result of the influence of cytostatic drugs on cell production.

Sternal puncture specimen analysis revealed compensatory shifts in the granulocytic lineage. Before the second course of chemotherapy, the content of mature neutrophilic granulocytes (MNG) significantly increased by more than 31% compared to the level before treatment (Fig. 1, *d*). Then, the number of both MNG and immature neutrophilic granulocytes increased by on average 15% from the initial level. It can be hypothesized that adriablastin/taxotere combination does not induce considerable disturbances in mitotically active granulocyte precursor cells, which provides the basis for activation of regenerative processes in the bone marrow at the early stages of cytostatic disease [2] and indirectly attests to progressive activation of not only proliferation but also differentiation of granulocytic lineage cells under the effect of these drugs.

This assumption is confirmed by considerable increase in the content of committed granulocytic precursors in the hemopoietic tissue due to activation of proliferation and differentiation of these cells as soon as after the first course of chemotherapy (Fig. 2). After the second course, the number of CFU-G remained elevated, but did not significantly differ from the initial level, which can be explained by exhaustion of the proliferative potential of precursor cells on the one side (Fig. 2, *b*) and by even more pronounced increase (by 1.6 times) in the index of maturation of granulocytic precursors (Fig. 2, *c*).

Massive release of bone marrow neutrophils into the circulation in response to glucorticoid administration is one of the most important functional peculiarities of these cells [7]. Administration of prednisolone to BC patients induced a transient increase in the number of peripheral blood neutrophils in the peripheral blood, which indicates the presence of a typical reaction to the hormone preparation (Table 1). Analysis of hormonal test parameters against the background of adriablastin/taxotere treatment revealed a high reserve potential of hemopoietic granulocytic lineage in the examined patients. For instance, before the second course of chemotherapy the release of neutrophils 2 h after prednisolone injection surpassed the corresponding parameter in patients before cytostatic treatment by 2.05 G/liter. The observed differences remained significant 5 and 6 h after prednisolone injection, while the maximum release surpassed the values observed in patients before chemotherapy by 2.13 G/liter. Hormonal test before the start of the third course showed further increase in neutrophil production, which surpassed the

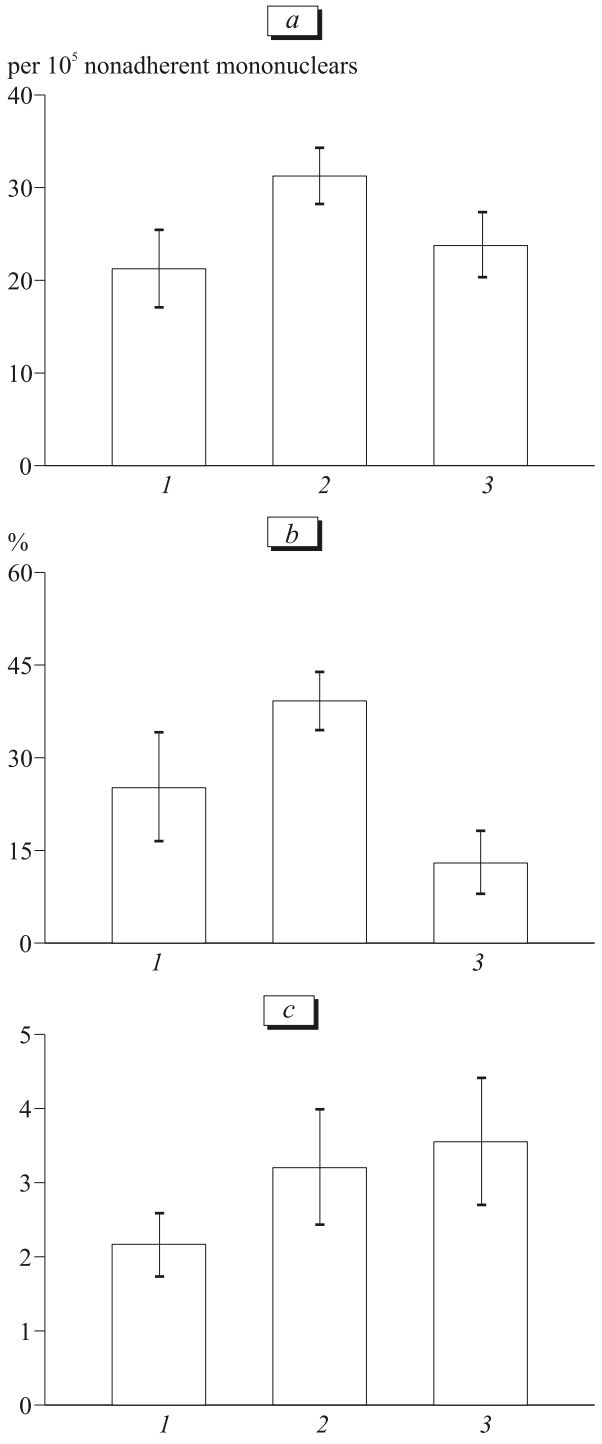

Fig. 2. Number of CFU-G (*a*), relative content of S-phase CFU-G (*b*), intensity of CFU-G maturation (*b*) in the bone marrow of patients with stage III-IV BC in the dynamics of antitumor therapy with adriablastin and taxotere. *1*) before treatment; *2*) after the first course; *3*) after the second course.

TABLE 1. Increase in Neutrophil Content (G/liter) and Their Maximum Release into Circulation in Response to Prednisolone Injection to Patients with Stage III-IV BC Treated with Adriablastin and Taxotere ($X \pm m$)

Parameter		Term of experiment		
		before treatment	before course 2	before course 3
Dynamics of the response	before injection	2.21±0.38	3.10±0.33	3.19±0.38
	2 h	3.88±0.63	5.93±0.72*	6.38±0.71*
	3 h	4.27±0.75	6.23±0.67	7.06±0.78*
	4 h	4.51±0.72	6.57±0.90	7.15±0.92*
	5 h	4.22±0.65	7.88±1.29*	6.88±0.82*
	6 h	3.80±0.70	7.05±1.03*	6.42±0.58*
Maximum release	G/liter	2.53±0.43	4.66±0.84*	5.42±0.60*
	%	116.80±17.06	136.60±23.15	181.50±21.55*

Note. *$p<0.05$ compared to the parameter before treatment.

level observed before therapy at all terms of the test. The maximum release surpassed the corresponding value in untreated patients by 2.9 G/liter (Table 1).

The positive dynamics of the presnisolon test is probably determined by accumulation of both morphologically differentiated cells and granulocyte precursors in the bone marrow and their enhanced proliferation and differentiation. The increase in the reserve capacities of the granulocyte lineage is probably related to the potentiating effects of dexamethasone, an obligatory component of premedication in case of taxane treatment [6] on the mobilizing effect of prednisolone.

Thus, we can conclude that accelerated maturation of hemopoietic precursors accumulated in the bone marrow due to their enhanced proliferation at the early stages of cytostatic disease plays an important role in maintaining hemopoiesis at a sufficient level and compensating for the toxic effects of chemotherapeutic drugs under conditions of cytostatic therapy with adriablastin and taxotere. Enhanced differentiation of these cells (even under conditions of suppressed proliferation) increased the count of morphologically discernible and mature elements of the bone marrow and peripheral blood and strengthened of the reserve potential of the bone marrow pool of neutrophil granulocytes.

Our data on the mechanisms underlying the changes in the blood system under the effect of the studied chemotherapy scheme explain good tolerability of the preparations, which is very important in view of extensive application of taxane-containing regimens of cytostatic therapy [8].

REFERENCES

1. M. L. Gershanovich, *Complication in Chemotherapy and Hormone Therapy of Malignant Neoplasms* [in Russian], Moscow (1982).
2. E. D. Gol'dberg, A. M. Dygai, and V. V. Zhdanov, *Role of Hemopoiesis-Inducing Microenvironment in the Regulation of Hemopoiesis in Cytostatic Myelosupressions* [in Russian], Tomsk (1999).
3. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
4. E. A. Kost, *Manual for Clinical Laboratory Tests* [in Russian], Moscow (1977).
5. G. F. Lakin, *Biometry* [in Russian], Moscow (1973).
6. L. V. Manzyuk and E. V. Artamonova, *Ros. Med. Zh. (Onkologiya)*, **14**, No. 24, 1753-1757 (2006).
7. D. Dale, A. Fauci, D. Guerry, and S. M. Wolff, *Clin. Invest.*, **56**, No. 4, 808-813 (1975).
8. D. Herceg and D. Vrbanec, *Lijec. Vjesn.,* **131**, Nos. 5-6, 133-141 (2009).
9. T. Ishikawa, S. Shimizu, N. Momiyama, *et al., Nippon Rinsho,* **64**, No. 3, 529-535 (2006).
10. J. M. Nabholtz and J. Gligorov, *Expert. Opin. Pharmacother.,* **6**, No. 7, 1073-1094 (2005).
11. J. M. Nabholtz and A. Riva, *Oncologist*, **6**, Suppl. 3, 5-12 (2001).