Compensatory Reactions during Impairment of Granulocytic Stem in Patients with Lung Cancer Receiving Antitumor Chemotherapy

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We compared changes in the granulocytic hemopoietic stem in patients with stage III-IV lung cancer receiving cytostatic therapy by the original CVC and standard CAM schemes. In patients treated with the CVC regimen, the granulocytic hemopoietic stem possessed more potent compensatory capacities.

Key Words: *chemotherapy; granulocytopoiesis; hemopoietic precursors; bone marrow neutrophil reserve*

Antitumor chemotherapy causes various side effects related to the toxic influence of cytostatics on intensively proliferating cells [1,3]. These complications include leukopenia with marked decrease in peripheral blood neutrophilic granulocyte (NG) count [1]. These cells protect the organism from infections and are involved in the development of antitumor resistance [5]. Therefore, drug combination and treatment regimens producing desired clinical effects and minimum side reactions are most preferable. Studies of the mechanisms underlying toxic effects of various chemotherapeutic regimens are of considerable importance.

Here we compared toxic effects of a new CVC chemotherapy and standard CAM scheme used in patients with lung cancer on the granulocytic hemopoietic stem. The mechanisms underlying different hematological effects of these regimens were studied.

MATERIALS AND METHODS

We examined 80 patients with stage III-IV lung cancer. Thirty-three patients received antitumor CVC chemotherapy: intravenous injections of 1.4 mg/m^2 vincristine, 600 mg/m^2 cyclophosphamide (day 1), and 300 mg/m^2 carboplatin (day 2). Forty-seven patients received CAM therapy: 750 mg/m^2 cyclophosphamide, 25 mg/m^2 adriamycin (days 1 and 8), and 20 mg/m^2 methotrexate (days 2 and 9).

The bone marrow and peripheral or venous blood were assayed.

Studies of peripheral blood parameters (leukocytes and hemogram) and differential bone marrow count in the sternal punctate were performed routinely [4]. Granulomonocytopoietic precursor cells (CFU-GM) were cloned from nonadherent karyocytes of the peripheral blood and bone marrow (2.0×10^5 cells/ml) in a semisolid nutrient medium containing 80% McCoy 5A medium, 19% fetal bovine serum, 280 mg/liter gentamicin (Serva), 0.4×10^5 M 2-mercaptoethanol (Sigma), and 5 μg/liter recombinant granulocyte colony-stimulating factor (G-CSF, Neipogen, Hoffman—La Rosche). The suspension (2 ml) was placed in 35-mm plastic Petri dishes and incubated at 37°C, 5% CO_2, and 100% humidity for 7 days. Colonies were counted in a Biolam-P 1 invertoscope (×56). Aggregates containing not less than 50 nuclear cells were considered as colonies. Morphology of individual colonies was

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assayed using preparations stained with azure II and eosin [2,10].

The bone marrow reserve of NG was evaluated by the prednisolone test [7]. Prednisolone (Gedeon Richter) in a dose of 60 mg was injected intravenously. The total leukocyte count and the content of segmented neutrophils were measured before and 2, 3, 4, 5, and 6 h after prednisolone administration. The results were analyzed by NG migration (% of initial count). The maximum release of neutrophils was expressed in percents and absolute values.

The results were analyzed by Student's *t* test [6].

RESULTS

Treatment of cancer patients according to both chemotherapeutic schemes decreased the total count of leukocytes after the first course of chemotherapy (Fig. 1, *a*). The total count of leukocytes slightly increased before the second course of CAM, but then dropped to a minimum. At the same time, during 2 courses of CVC chemotherapy the total leukocyte count progressively decreased and leukopenia was more pronounced than in patients receiving CAM (Fig. 1, *a*).

Leukopenia was related to a decrease in the content of peripheral blood lymphocytes and segmented granulocytes (Fig. 1, *c*, *d*). However, the content of stab neutrophils in patients treated according to CVC scheme was much higher than in patients receiving CAM (Fig. 1, *b*).

These results indicate that CVC therapy is accompanied by rapid migration of immature NG from the bone marrow, which reaches a maximum after the second course of chemotherapy. In patients receiving CVC therapy the content of stab and segmented neutrophils in the bone marrow was lower than that in patients receiving CAM and than in the control (by 25%). By contrast, the content of immature NG (myeloblasts, promyelocytes, myelocytes, and metamyelocytes) in CVC-treated patients was much higher than in patients given CAM. This probably indicates that CVC scheme produced minor effects on mitotic pre-

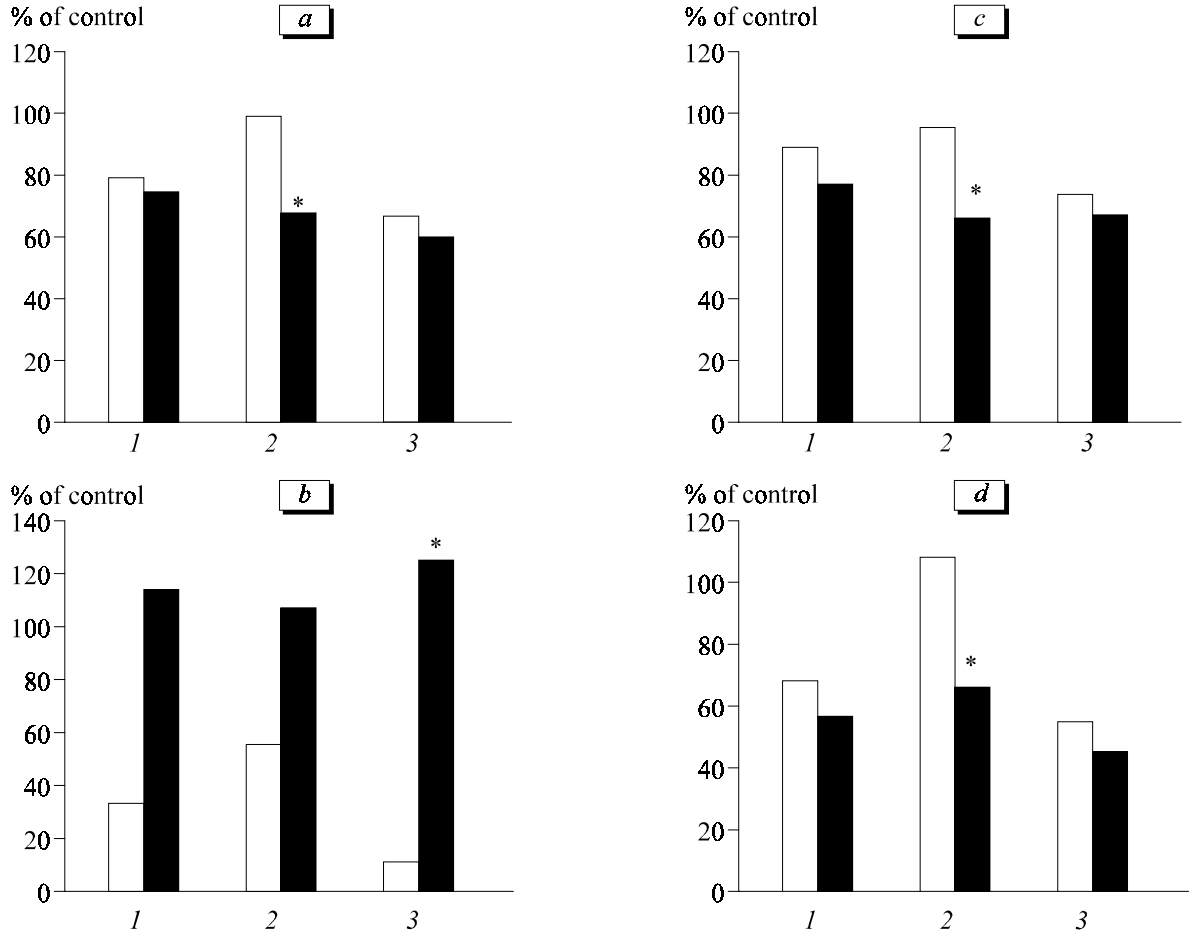

Fig. 1. Total count of peripheral blood leukocytes (*a*), stab (*b*) and segmented neutrophils (*c*), and lymphocytes (*d*) in patients with stage III-IV lung cancer after the first (*1*), before the second (*2*), and after the second course (*3*) of CAM (light bars) or CVC chemotherapy (dark bars). Here and in Figs. 2 and 3: *$p<0.05$ compared to CAM.

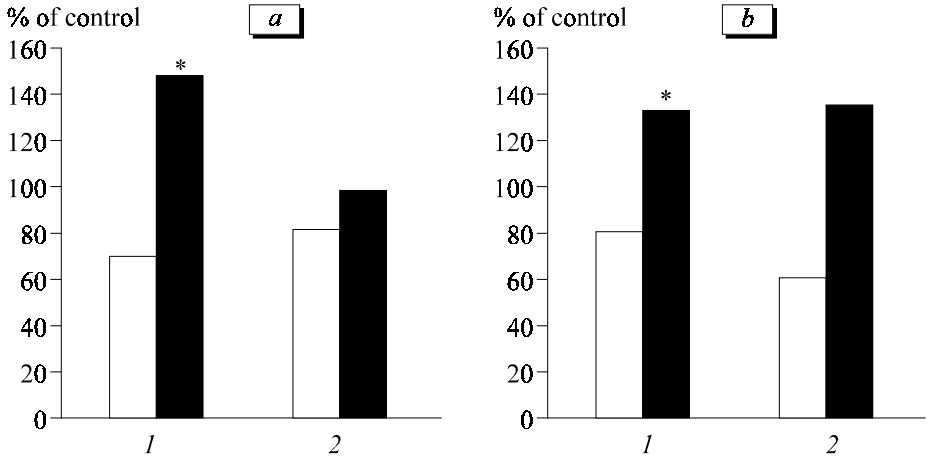

Fig. 2. Count of committed granulomonocytic precursors in the bone marrow (*a*) and peripheral blood (*b*) in patients with stage III-IV lung cancer after the first (*1*) and second courses (*2*) of CAM (light bars) or CVC chemotherapy (dark bars).

cursors, which contributes to activation of regenerative processes in the bone marrow during the early stage of cytostatic disease [8].

This assumption is confirmed by a considerable increase in the content of committed granulomonocytic precursors in the hemopoietic tissue after the first course of CVC chemotherapy (Fig. 2, *a*). After the second course of chemotherapy this parameter remained high, but did not differ from the initial level. This is probably related to the exhaustion of compensatory mechanisms providing regeneration of hemopoiesis (Fig. 2, *a*).

In patients receiving CAM therapy, the count of bone marrow CFU-GM decreased and significantly differed from that in patients receiving CVC (Fig. 2, *a*). Moreover, changes in the count of peripheral blood hemopoietic precursors in patients treated by different schemes were different. During CVC therapy, the count of granulomonocytic precursors remained high until the third course of chemotherapy. By contrast, in patients treated according to the CAM protocol the count of circulating CFU progressively decreased and differed from the initial level and from that in patients receiving CVC chemotherapy (Fig. 2, *b*).

The absence of migration of CFU-GM into the circulation in patients receiving CAM therapy was probably associated with cytostatic-induced disturbances in mobilization of clonogenic elements and exhaustion of their bone marrow reserves. Thus, migration of hemopoietic precursors from the bone marrow is suppressed during CAM treatment.

One of the most important properties of mature bone marrow neutrophils is their intensive migration into the circulation induced by glucocorticoids. In our experiments, prednisolone transiently increased the count of peripheral blood neutrophils in patients of

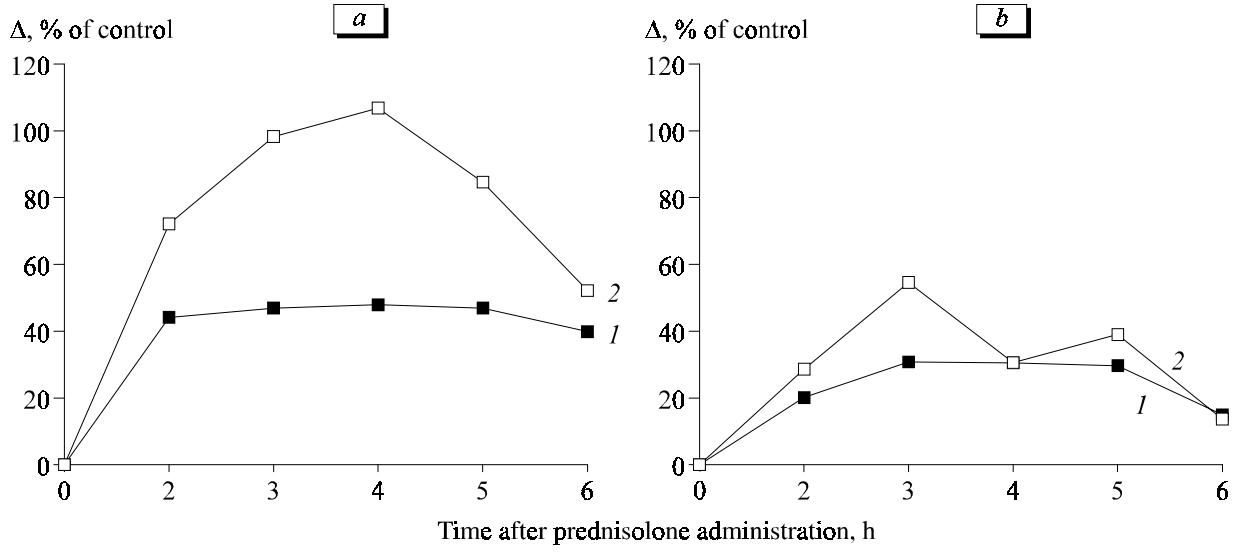

Fig. 3. Neutrophil count in patients with stage III-IV lung cancer after the first (*1*) and second courses (*2*) of CAM (light bars) or CVC chemotherapy (dark bars).

both treatment groups. Therefore, bone marrow neutrophils adequately reacted to the hormone (Fig. 3). Leukocytosis was more pronounced after the first and second CVC courses (Fig. 3, *a*, *b*). During CVC therapy, the increase in peripheral blood neutrophil count caused by prednisolone 2-fold surpassed that after the first course of CAM chemotherapy. These differences were significant over 5 h after prednisolone administration (Fig. 3, *a*). After the first course of CVC chemotherapy the maximum release of neutrophils in response to pharmacological stimulation was higher than in patients receiving CAM (4.75±0.85 and 1.88± 0.19 g/liter, respectively).

However, in patients receiving CVC therapy, migration of NG also decreased by the end of the second course. Therefore, no differences between these groups of patients were found (Fig. 3, *b*).

The response to prednisolone depends on the count of bone marrow NG [9]. Our findings suggest that CVC therapy causes less pronounced disturbances in proliferating NG. It should be emphasized that the recovery of bone marrow neutrophil reserves primarily depends on these cells.

Our findings indicate that in patients receiving CVC therapy, the granulocytic hemopoietic stem possesses more potent compensatory capacity during cytostatic-induced damages. This is related to intensive functioning of the central granulocytopoietic stem, adequate reactions of the bone marrow and circulating precursor cells to hemopoietic tissue damages, and greater reserve capacities of bone marrow mature neutrophils at the early stage of CVC treatment (compared to CAM scheme).

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