Reaction of Bone Marrow Hematopoiesis to the Toxic Effect of Paclitaxel

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Intraperitoneal injection of paclitaxel (Mitotax) in a single dose of 40 mg/kg was followed by an increase in the number of mitotic granulocytic and erythroid cells, hypoplasia, and pancytopenia of the bone marrow in CBA/CaLac mice. The test preparation decreased the number of hematopoietic precursor cells for erythropoiesis and granulocytopoiesis, but increased the count of polyploid cells and incidence of structural and genomic abnormalities in bone marrow cells. These changes were reversible.

Key Words: *paclitaxel; bone marrow; hematopoietic precursor cells; myelotoxicity; genotoxicity*

Paclitaxel (Taxol, Mitotax) is extensively used in medical practice during the last decades. This drug is used for the therapy of ovarian cancer, lung cancer, breast cancer, head tumors, and neck tumors [1,2,7,8,15]. The potent antitumor effect of paclitaxel is related to the inhibition of cell mitotic activity. Paclitaxel prevents reorganization of the microtubular network, which is important for normal cell function. Paclitaxel stabilizes the arrangement of microtubules from tubulin dimers and suppresses their depolymerization [9,10]. These processes lead to cell apoptosis [11].

Various side effects that develop after taxane chemotherapy, including paclitaxel were reported. Taxane therapy is accompanied by neurotoxicity, cardiotoxicity, and drug hypersensitivity [1,5,7,8]. Dose-limiting toxicity of the drug is associated with neutropenia, thrombocytopenia, and anemia [2,7]. These changes increase the risk of infection and lengthen the period of hospital treatment due to neutropenia and fever [12]. Little is known about the mechanisms for myelotoxic action of paclitaxel (particularly damage to hematopoietic organs). Here we studied the reaction of hematopoietic organs to paclitaxel treatment.

MATERIALS AND METHODS

Experiments were performed on 110 CBA/CaLac mice (class I conventional strain) obtained from the nursery of the Institute of Pharmacology (Tomsk Research Center). The animals were maintained according to the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The mice received single intraperitoneal injection of Mitotax (paclitaxel, Dr. Reddy's Laboratories Ltd) in a maximum tolerated dose of 40.0 mg/kg. This dose of paclitaxel was estimated by probit analysis for 30 days. Control animals intraperitoneally received an equivalent volume of physiological saline (solvent). Peripheral blood (from 5 animals of each group) was analyzed 6, 24, and

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48 h and 4, 7, 9, 11, and 14 days after paclitaxel injection on an Abacus automatic analyzer (Diatron) operated in veterinary regimen. The following parameters were estimated: hemoglobin concentration (HGB, g/liter), erythrocyte count (RBC, 10^{12}/liter), hematocrit (HCT, %), platelet count (PLT, 10^9/liter), total leukocyte count (WBC, 10^9/liter), and differential leukocyte count. Reticulocytes were counted in blood smears after supravital staining with brilliant cresyl blue [3]. The animals were euthanized by cervical dislocation under ether anesthesia. The total number of bone marrow cells and parameters of myelogram were estimated (10^6 per femur). Morphological examination of cells from the peripheral blood and bone marrow (leukogram and myelogram) included microscopic study of cytological preparations that were stained with azure II and eosin (Nocht's method) [3]. Cytogenetic abnormalities were studied 6, 24, and 48 h and 5 and 14 days after Mitotax injection. The state of chromosomes on metaphase plates of the bone marrow (number of abnormal metaphases, chromosomal aberrations, chromatid fragment, and exchanges per 100 cells; count of cells with gaps) was estimated by the method of Ford with modifications [6]. The number of micronucleated erythrocytes (‰) was evaluated in the peripheral blood smear by analyzing 5000-6000 erythrocytes [4].

The content of granulocyte colony-forming units (CFU) and cluster-forming units (ClFU), granulomonocytic CFU (CFU-GM), fibroblast colonies (CFU-F), and erythroid CFU (CFU-E) and ClFU (ClFU-E) in the bone marrow was estimated by *in vitro* cloning of myelokaryocytes in the methylcellulose culture medium [3]. Differentiation of erythroid and granulocytic precursors was assayed by the index of maturation (cluster/colony ratio in the well) [3].

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by parametric Student's test and nonparametric Mann—Whitney test.

TABLE 1. Number of CFU-GM and CFU-F in the Bone Marrow of CBA/CaLac Mice after Mitotax Injection (per 10^5 myelokaryocytes, $X \pm m$)

Period of study	CFU-GM	CFU-F
Control	0	2.0±0.5
6 h	0.2±0.2	0.3±0.2*
24 h	2.3±0.7*	0.7±0.2
48 h	1.2±0.3*	1.0±0.4
Day 5	4.2±0.7*	7.5±0.9*
Day 9	6.5±0.6*	7.2±0.9*
Day 14	0	1.5±0.3

Note. Here and in Table 2: *$p<0.05$ compared to the control.

RESULTS

Single intraperitoneal injection of Mitotax was followed by a significant decrease in the total number of nucleated cells in mouse bone marrow (Fig. 1, *a*). The absolute number of immature and mature neutrophilic leukocytes in the bone marrow decreased 24 and 48 h after Mitotax injection. The count of mature neutrophils decreased on days 5, 9, and 11 after treatment (Fig. 1, *b*, *c*). The number of erythroid cells in Mitotax-receiving mice was lower than in control animals (Fig. 1, *e*). Mitotic activity of the granulocytic and erythroid hematopoietic stem significantly increased 6 h after Mitotax injection. Mitotic activity of the granulocytic stem decreased after 24 and 48 h. Mitotic activity of the erythroid stem decreased 48 h and 9 days after treatment (Fig. 1, *d*, *f*). The count of bone marrow lymphocytes sharply decreased 6, 24, and 48 h and 14 days after Mitotax injection. The absolute number of segmented neutrophilic leukocytes and platelets in the peripheral blood decreased after 24 and 48 h (Fig. 1, *g*). The number of peripheral blood lymphocytes remained below the normal in all periods of study. Examination of red blood cells showed that the number of reticulocytes decreased 24

TABLE 2. Cytogenetic Effects of Mitotax in MTD on CBA/CaLac Mice ($X \pm m$)

Period of study	Chromosomal aberrations, %	Polyploid cells, %	Cells with gaps, %	Micronucleated erythrocytes, ‰
Control	0.71±0.35	0.00±0.00	0.71±0.56	1.38±0.14
6 h	1.23±0.40	0.57±0.3*	1.50±0.90	3.41±0.87*
24 h	5.32±2.40*	4.99±1.54*	2.52±0.81	2.31±0.23*
48 h	2.69±1.71	15.58±2.02*	2.66±1.69	1.40±0.22
Day 5	0.72±0.43	2.77±0.39*	1.62±0.44	1.67±0.10*
Day 14	0.25±0.25	1.25±0.75*	2.19±0.63	1.98±0.24

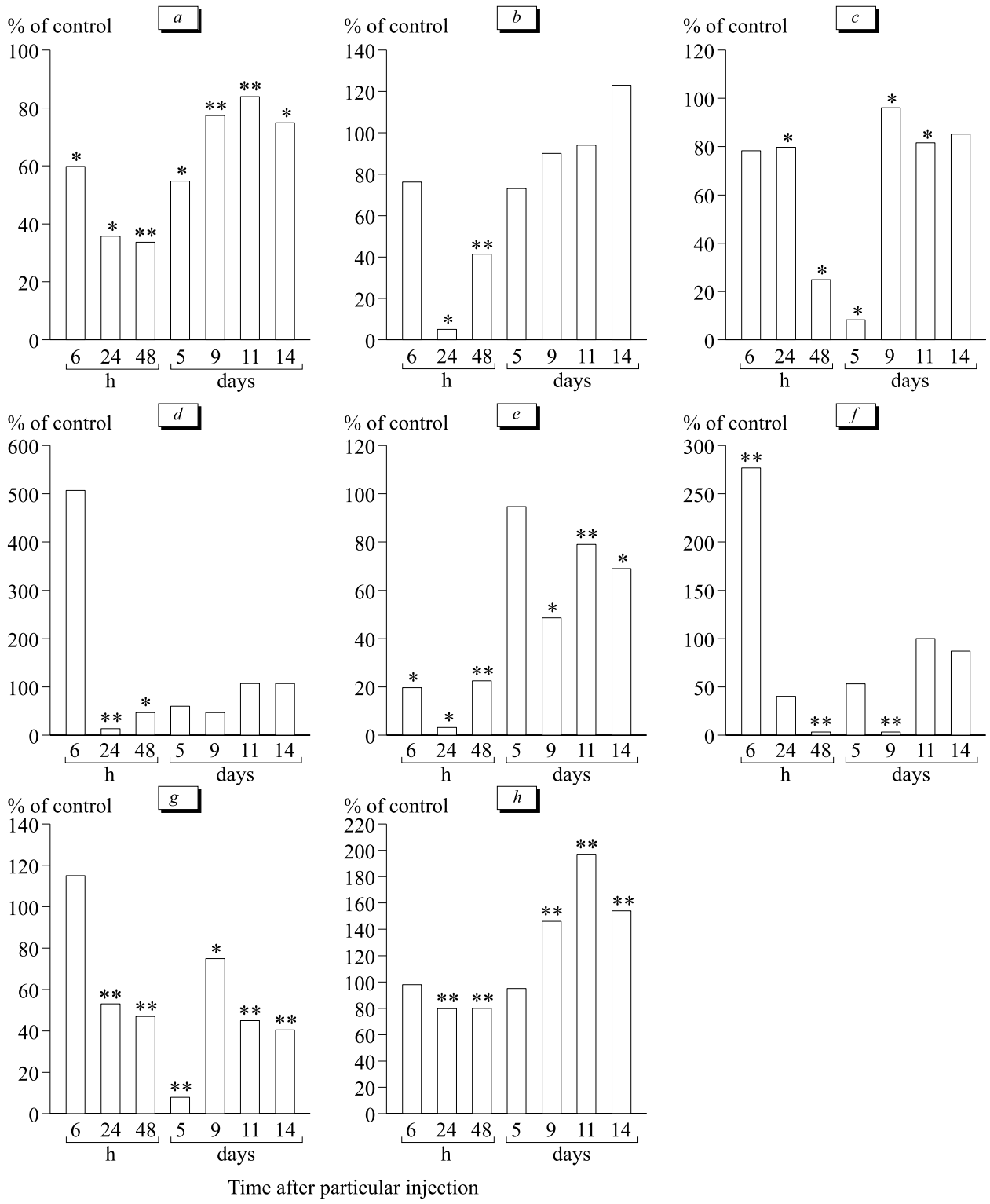

Fig. 1. Total number of myelokaryocytes (*a*), immature (*b*) and mature neutrophilic granulocytes (*c*), mitotic granulocytic cells (*d*), erythronormoblasts (*e*), and mitotic cells of the erythroid hematopoietic stem in the bone marrow (*f*) and count of mature neutrophilic leukocytes (*g*) and reticulocytes in the peripheral blood (*h*) of CBA/CaLac mice after paclitaxel injection. Here and in Fig 2: $^{*}p<0.05$ and $^{**}p<0.01$ compared to the control.

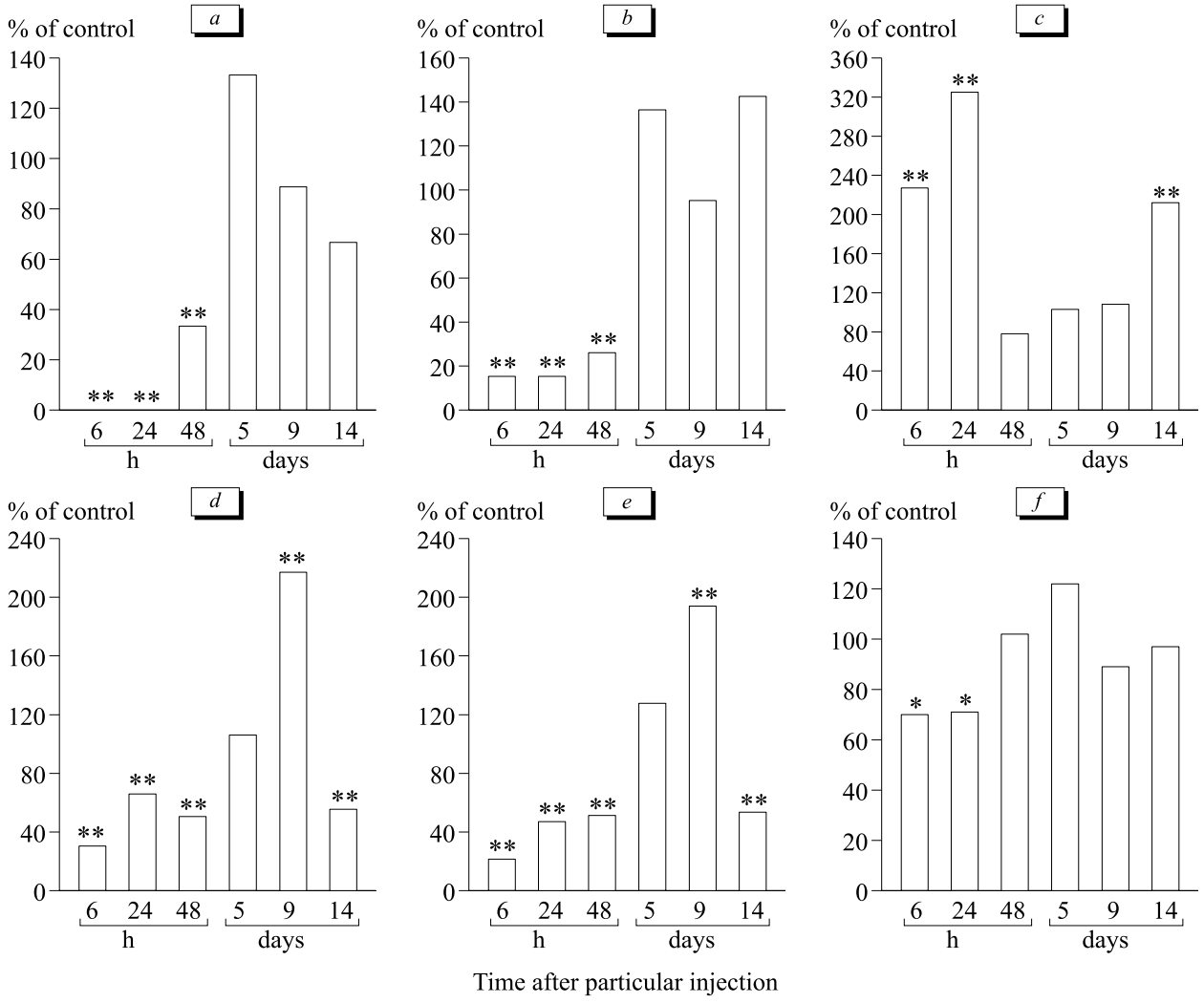

Fig. 2. Content of erythrocyte colony-forming units (*a*) and cluster-forming units (*b*), maturation index of erythroid precursors (*c*), content of granulocyte colony-forming units (*d*) and cluster-forming units (*e*), and maturation index of granulocytic precursors (*f*) in the bone marrow of CBA/CaLac mice after paclitaxel injection.

and 48 h after Mitotax injection, but increases on days 9, 11, and 14. The increase in reticulocyte count was accompanied by a decrease in hemoglobin concentration (all period of study), erythrocyte number (except for 6 and 48 h postinjection), and hematocrit (24 and 48 h and 11 days after Mitotax injection).

Mitotax-induced changes in the cellularity and morphological composition of the bone marrow were associated with the state of granulocytopoiesis and erythropoiesis precursor cells. For example, the number of colony-forming and cluster-forming erythropoiesis precursor cells decreased after 6, 24, and 48 h (Fig. 2, *a*, *b*). Differentiation of erythroid precursors significantly exceeded the control level 6 and 24 h and 14 days after Mitotax injection (Fig. 2, *c*).

The number of granulocytic precursors in the bone marrow of Mitotax-treated mice was below normal after 6, 24, and 48 h and 14 days (Fig. 2, *d*, *e*). Differentiation of these cells was suppressed only 6 and 24 h after Mitotax injection (Fig. 2, *f*).

Variations in the number of granulomonocytic precursors in the bone marrow of mice differed from that of granulocytic precursors. The amount of granulomonocytic CFU increased after 24 and 48 h and 5 and 9 days (Table 1). The formation of fibroblast colonies from precursors of stromal mechanocytes decreased 6 h after Mitotax injection (Table 1). Migration of CFU-F from the bone marrow significantly increased on days 5 and 9 after Mitotax injection (Table 1).

Studying the effect of cytogenetic drugs on the bone marrow revealed a significant increase in structural abnormalities of chromosomes at metaphase plates 24 h after Mitotax injection. It was mainly related to chromatid breaks. In other experiments,

the incidence of structural chromosome abnormalities did not differ from the control (Table 2). The ratio of polyploid cells (4n) significantly increased 6 h after Mitotax treatment and reached maximum 48 h postinjection. The number of micronucleated erythrocytes in the peripheral blood significantly increased 6 h after paclitaxel injection and remained unchanged for 14 days. Microscopy of blood smears revealed the presence of erythrocytes with large micronuclei. The appearance of these cells is associated with changes in cell division (*i.e.* genomic abnormalities) [4].

Our results indicate that suppression of mitotic activity of granulocytic and erythroid cells in the bone marrow (G_2/M phase) [7] and hypoplasia of the bone marrow in CBA/CaLac are observed over the first hours after intraperitoneal injection of Mitotax (paclitaxel) in a single dose of 40 mg/kg. Study of the peripheral blood revealed moderate pancytopenia, hypoplastic anemia, short-term severe neutropenia, moderate lymphocytopenia, and thrombocytopenia. Changes in morphological and quantitative characteristics of the bone marrow are closely related to the state of hematopoietic and mesenchymal precursors under conditions of myelosuppression. Mitotax significantly decreased the number of colony-forming and cluster-forming precursor cells for erythropoiesis and granulocytopoiesis, but had little effect on granulomonocytic and fibroblast precursors. Mitotax increased the number of micronucleated erythrocytes in the peripheral blood and incidence of structural and genomic abnormalities in bone marrow cells. These data show that Mitotax modulates dynamic reorganization of the microtubular network, which is important for normal cell function [9,10]. Single intraperitoneal injection of Mitotax induces moderate mitogenic, mutagenic, and apoptogenic effects and causes reversible hypoplasia of the bone marrow, moderate hypoplastic anemia, thrombocytopenia, and neutropenia.

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