Reaction of the Blood System in Pregnant Rats at Late Terms after Etoposide Treatment

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Supplement 1, pp. 75-78, January, 2007
Original article submitted November 11, 2006

Parameters of peripheral blood in female rats remained practically unchanged at late terms after single treatment with antitumor drug etoposise. Pregnancy was accompanied by the development of reversible regeneration anemia. Proliferative activity of bone marrow erythronormoblasts decreased.

Key Words: *etoposide; rats; pregnancy; blood system*

The number of women in long-term complete remission after chemotherapy venturing on pregnancy grows in recent years [12,13]. Since all antitumor drugs are highly toxic compounds, there is high probability of various pathologies in children born by these women. It was previously demonstrated that toxic effect of etoposide on the progeny is more potent, when the drug is administered to female rats compared to its administration to males [3]. This can be explained by higher sensitivity of female sex cells to the cytostatic treatment compared to male sex cells. The existence of delayed effects of the toxic action of antitumor preparations on normal (non-tumor) cells of the maternal organism cannot also be excluded.

Suppression of hemopoiesis is a regular consequence of any cytostatic treatment. It can persist for a long time without considerable changes in the parameters of peripheral blood (PB) cells [5,8]. In case of pregnancy producing additional strain in the hemopoietic system, the hematotoxic effects of antitumor preparations can be more pronounced. Hemolytic anemia at late terms after treatment with cytostatic carboplatin was observed in pregnant females, but not in non-pregnant rats [7].

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The aim of the present study was to compare PB system in pregnant rats at late terms after treatment with antitumor drug etoposide, a topoisomerase inhibitor. This preparation was chosen because it is widely used in oncology [9] and its myelotoxicisity is a dose-limiting factor [2]. Moreover, the toxic effect of etoposide on the progeny is more potent, when the drug is administered to female rats compared to its administration to males [1].

MATERIALS AND METHODS

Experiments were carried out on 30 female Wistar rats of reproductive age weighing 250 g. The animals were obtained from the Laboratory of Biomedical Modeling, Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. The animals were kept in accordance with European Convention on Protection of Vertebrate Animals Used in Experimental and Scientific Purposes (Strasbourg, 1986). Experimental female rats (n=15) received single intravenous injection of etoposide (vepesid, Teva) in MTD (30 mg/kg) calculated by the method of graphic probit-analysis from the data obtained during 30-day observation of treated animals [9]. Control rats (n=15) received an equivalent volume of vehicle (physiological saline). One, 3, and 6 months after treatment the experimental and control female

0007-4888/07/Supp-064

rats were mated with intact males. Mating was confirmed by vaginal smears [10], pregnancy was verified by autopsy. PB parameters in control and experimental groups were analyzed before pregnancy (control 1 and experiment 1) and on day 20 of pregnancy (control 2 and experiment 2). PB was taken from the caudal vein after amputation of the tail tip. Blood cells were analyzed on an Abacus hematological analyzer (Diatron) in veterinary regimen [6]. Reticulocytes were counted in blood smears after supravital staining with brilliant cresyl blue [6]. On day 20 of pregnancy, the rats were sacrificed by cervical dislocation and bone marrow parameters were analyzed. Leuko- and myelograms were analyzed by microscopy of smears fixed with absolute methanol and stained by the method of Nocht—Maksimov [6].

The data were processed statistically using the Student and Wilcoxon—Mann—Whitney tests.

RESULTS

In non-pregnant female rats (experiment 1), the majority of studied PB parameters did not differ from the corresponding control values 1 and 3 months after etoposide treatment (Table 1). The only exclusion was reticulocyte count, which was higher in this groups compared to intact animals. Six months after cytostatic exposure, the total number of platelets and lymphocytes significantly decreased, while the content of neutrophilic granulocytes and monocytes increased.

In pregnant rats (control 2), the studied parameters did not significantly differ from those in intact rats (control 1, Table 1). In pregnant rats receiving etoposide (experiment 2) 1 month before mating, the mean values of hematocrit, erythrocyte count, hemoglobin content, erythrocyte volume, and platelet count decreased compared to the corresponding parameters in intact pregnant rats. The

TABLE 1. PB Parameters in Pregnant Rats in Delayed Terms after Etoposide Treatment ($X \pm m$)

Parameter	Term of mating after etoposide treatment, months					
	1		3		6	
	control 1/2	experiment 1/2	control 1/2	experiment 1/2	control 1/2	experiment 1/2
Hemoglobin, g/liter	132.95±9.35	126.40±9.12	142.40±5.79	133.80±3.02	130.00±9.32	121.80±4.72
	132.00±6.04	110.00±11.12**	134.00±31.89	160.40±6.29^{+}	153.50±12.18	142.67±12.25
Erythrocytes, 10^{12}/liter	7.59±0.60	7.13±0.52	8.38±0.35	8.20±0.29	7.38±0.59	7.15±0.22
	7.73±0.48	5.78±1.19**	9.23±1.08	10.01±0.57	8.67±0.96	7.70±0.47
Hematocrit, %	38.78±2.74	36.56±2.44	35.52±7.55	38.88±1.00	37.80±3.36	36.44±1.31
	38.04±2.26	28.13±5.57**	40.17±4.26	49.45±3.63**	42.30±3.36	39.00±3.13
Mean erythrocyte volume, fl	51.40±0.75	51.40±0.11	49.60±0.68	47.60±0.51	51.00±0.89	51.00±0.55
	49.80±0.20	48.75±0.28**$^{+}$	52.67±0.33	49.25±0.25**	48.00±0.41	51.00±0.58**
Anisocytosis of erythrocytes, %	16.68±0.35	16.58±0.37	16.28±0.21	16.18±0.11	15.82±0.21	15.78±0.07
	16.82±0.26	16.56±0.19	16.84±0.22	17.30±0.32	14.65±1.04	15.40±0.91
Reticulocytes, %0	23.80±2.10	36.40±3.50*	19.60±4.20	34.80±6.18*	33.40±7.23	39.80±3.51
	29.80±3.87	26.33±1.86	23.00±6.72	26.0±0.87	22.00±3.92	50.33±5.78**
Platelets, 10^{9}/liter	865.2±78.0	702.0±81.6	835.2±71.5	828.0±56.7	1062.8±167.8	681.0±84.7*
	1005.0±100.0	643.3±180.6**	642.4±77.2	776.2±82.0	1032.2±209.1	1087.0±214.9
Leukocytes, 10^{9}/liter	10.54±0.97	12.88±0.99	11.00±1.20	9.86±0.90	8.98±1.45	6.62±0.95
	9.61±0.98	8.40±3.22	12.31±1.15	14.25±1.81^{+}	9.48±2.55	15.71±3.17**$^{+}$
Neutrophils, %	20.40±5.56	14.60±2.73	17.60±2.29	24.75±2.17	19.00±3.15	28.40±4.79*
	32.40±3.30	38.67±9.21	19.60±5.40	24.75±2.17	19.25±7.48	22.33±4.67
Lymphocytes, %	67.80±5.93	69.60±2.62	69.40±2.36	62.25±2.90	71.60±4.80	58.80±3.71*
	58.80±2.33	43.67±7.88**$^{+}$	64.00±4.04	62.25±2.90	65.00±8.69	53.00±5.51
Monocytes, %	10.20±0.92	13.00±1.64*	10.20±0.86	10.00±2.12	7.20±1.16	11.40±1.21*
	8.20±1.65	14.67±0.33**$^{+}$	8.33±2.73	10.00±2.12	14.00±2.94	21.67±1.76**$^{+}$

Note. $p \leq 0.05$ compared to: *control 1, **control 2, $^{+}$experiment 1.

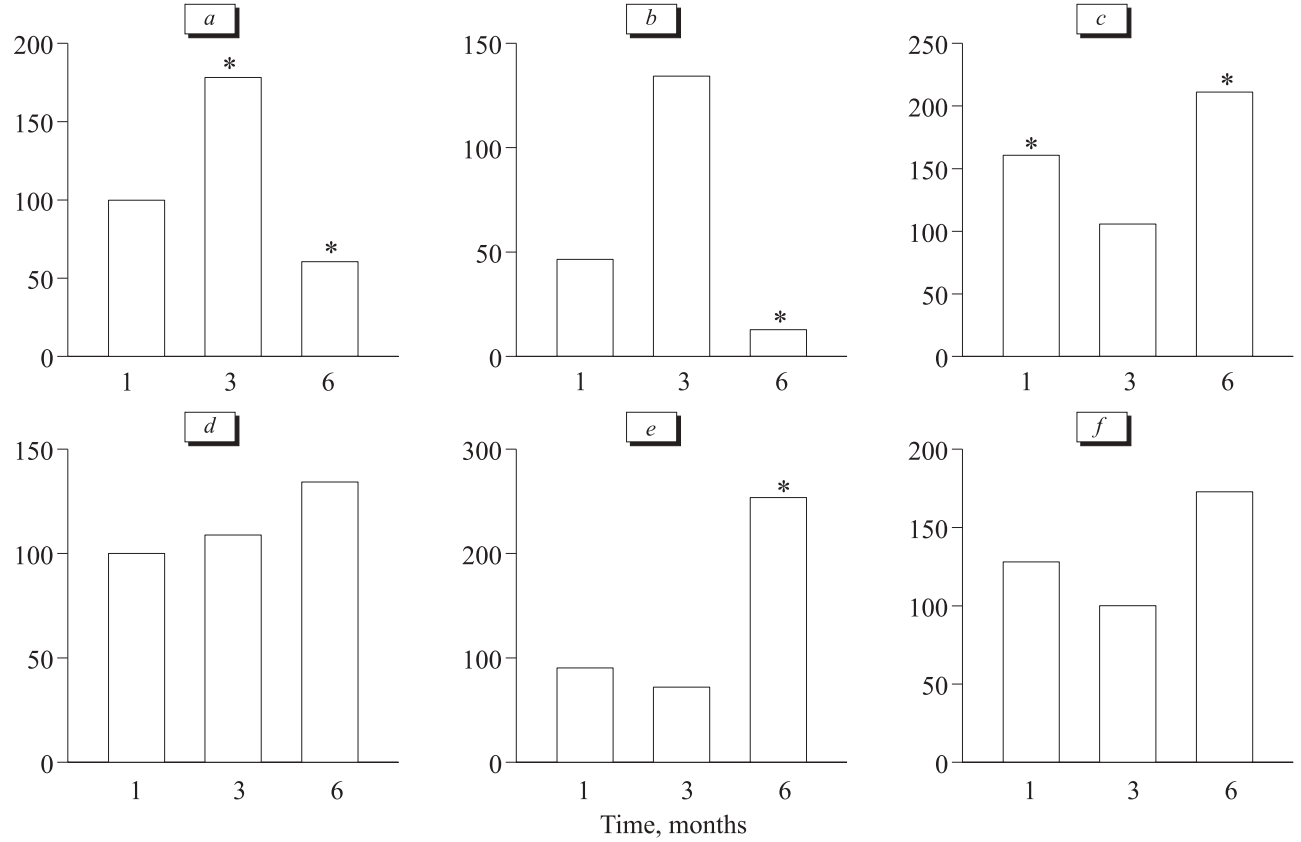

Fig. 1. Total number of erythronormoblasts (*a*), mitoses in erythroid cells (*b*), immature (*c*) and mature (*d*) neutrophil granulocytes, eosinophils (*e*), and monocytes (*f*) in the bone marrow of pregnant rats at delayed terms after etoposide treatment compared to the control (100%). *$p \leq 0.05$ compared to the control.

total number of lymphocytes also decreased, while the number of monocytes increased compared to those in control 2 and to those before pregnancy (experiment 1). These changes are typical of strained erythropoiesis. It is obvious, that these processes are determined by high sensitivity of the erythroid hemopoietic stem to etoposide [4]. It cannot be excluded that the observed effect results from decreased lymphocyte count, because T cells and their lymphokines are known to regulate erythropoiesis [14,15]. The pronounced monocytic reaction is also typical of etoposide [4]. The observed changes in the mean parameters of PB cells were not accompanied by suppression of the erythroid hemopoietic stem in the bone marrow, but granulocytopoiesis was activated due to the increase in the number of immature neutrophil granulocytes (Fig. 1). This attests to recovery of bone marrow hemopoiesis suppressed at early terms after etoposide treatment [4].

In pregnant rats (experiment 2), hematocrit and mean erythrocyte volume increased 3 months after the start of the experiment compared to the corresponding values in intact pregnant rats (control 2), the mean hemoglobin content and the total leukocyte count increased compared to the corresponding parameters in non-pregnant females receiving etoposide (experiment 1). The absolute number of erythronormoblasts in the bone marrow significantly increased. These data indicate increased regeneration capacity of the bone marrow, primarily of the erythroid stem, after its suppression in experimental rats observed 1 month after the start of the experiment.

In pregnant rats receiving etoposide 6 months before mating (experiment 2), the mean erythrocyte volume and the number of reticulocytes in PB increased compared to those in intact pregnant rats (control 2). The total number of leukocytes and monocytes increased compared to control 2 and values before pregnancy. In the bone marrow, the number of erythronormoblasts and mitoses in these cells decreased, while the content of immature granulocyte forms and eosinophils increased.

Mild changes in the studied parameters of PB observed in nonpregnant rats 1, 3, and 6 months after etoposide treatment attest to minor delayed aftereffects of the toxic action of this drug on parameters of hemopoiesis, which agrees with published data [8]. Under conditions of additional strain of hemopoiesis (pregnancy), etoposide treatment induced the development of regeneration anemia.

The pattern of changes in PB (decreased erythrocyte count, hemoglobin content, hematocrit) and bone marrow (hyperplasia of the erythroid hemopoietic stem) as well as the mechanism underlying the effect of etoposide on the erythron [4] drove us to a conclusion on hemolytic nature of the observed anemia. The pronounced increase in erythropoiesis regeneration capacity 3 months after etoposide treatment was a compensatory reaction to superinducing influence of the cytostatic and after 6 months we observed a decrease in the number of erythroid elements and inhibition of their proliferation in the bone marrow.

It is known that anemia produces a negative impact on intrauterine development and the course of early neonatal period [11]. In light of this, the observed toxic effect of etoposide on the blood system in pregnant rats can determine its previously described toxic effect on the progeny [1].

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