LATE REACTIVITY OF THE BLOOD SYSTEM AFTER INJECTION OF THE ANTITUMOR ANTIBIOTIC RUBOMYCIN

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Clinical observations and experimental data in recent years [3-6] have confirmed the validity of theortical suggestions that drugs with a cytostatic mechanism of action may cause late damage to hematopoiesis. It has been shown, in particular [1, 2], that disturbances of hematopoietic function due to cytostatics may remain latent and compensated for a long time, without reflection in quantitative and morphological parameters of the blood cells, and they become manifest only in a situation causing additional disturbance of hematopoiesis.

The aim of the present investigation was an experimental study of the functional and compensatory capacity of granulocytopoiesis and erythropoiesis 1, 3, and 6 months after injection of anitumor anthracycline antibiotics (with particular reference to the "classical" substance of this group, rubomycin), which currently occupy a leading place in the arsenal of antitumor drugs available for use in clinical practice.

EXPERIMENTAL METHOD

Experiments were carried out on 206 noninbred rats weighing 160-220 g and on 120 mature BALB/c mice. Of the total number of animals 65 rats and 60 mice served as the control. Rubomycin was dissolved in sterile physiological saline immediately before intraperitoneal injection in a dose of 1.5 mg/kg (calculated by the graphic method of probit analysis, and corresponding to the therapeutic sessional dose) daily for 10 days. Parameters of the peripheral blood and bone marrow were studied by standard hematologic methods. The state of the hematopoietic stem cell pool (CFU_c) was investigated by the exogenous cloning method [7] in a lethally irradiated (7.5 Gy) syngeneic recipient. The powers of compensation of the granulocytic series of hematopoiesis were estimated from the acute leukocytic reactions to injection of drugs mobilizing neutrophils from the marginal pool (15 and 30 min after a single subcutaneous injection of a 0.1% solution of adrenalin hydrochloride in a dose of 0.5 ml/m^2) and from the granulocytic reserve of the bone marrow (during the first 6 h after intraperitoneal injection of hydrocortisone in a dose of 15 mg/kg). The effect of rubomycin on neutrophilic function was judged by the phagocytic reaction and activity of the principle enzymes involved in the final phase of phagocytosis (myeloperoxidase, acid phophatase). The compensatory capacity of the erythron was studied on a model of acute measured blood loss, amounting to 2% of the body weight (up to 30-40% of the circulating blood volume) from the femoral vein, and the functional capacity of the bone marrow was estimated 2, 3, 5, 10 and 15 days after the development of anemia, on the basis of the following criteria: the total number of nucleated cells, the absolute number of erythronormoblasts, and the number of stathmokinetic mitoses in them.

EXPERIMENTAL RESULTS

The experiments showed that 1, 3 and 6 months after the end of the course of rubomycin injections the pool of hematopoietic stem cells and the morphological composition of the bone marrow and peripheral blood were completely restored. Meanwhile (Table 1) normalization of the functional parameters of neutrophilic leukocytes was not observed until the 6th month of observation, and after 1 and 3 months the animals still exhibited marked disturbances of the phagocytic function of the neutrophils, expressed mainly as inhibition of the final phase of phagocytosis, and evidently due to reduced activity of the key enzymes of the granulocytes.

The results of the adrenalin test indicated complete exhaustion of the marginal pool of

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TABLE 1. Functional Characteristics of Peripheral Blood Neutrophils of Rats 1, 3, and 6 Months After End of Course of Rubomycin Injections in Dose of 1.5 mg/kg Body Weight (X ± m)

Parameter studied	Time of investigation, months				
	Control	1	3	Control	6
PAL, %	77,4±1,8	78,4±3,3	59,5±9,4	51,6±9,2	48,3±6,1
P		>0,5	<0,05		>0,5
IC	5,3±0,4	5,9±0,4	9,6±0,8	7,6±1,7	11,4±0,4
P		>0,5	<0,001		>0,05
ICP	58,9±2,1	35,5±1,7	48,6±1,2	55,0±2,0	48,8±2,4
P		<0,001	<0,001		>0,05
Myeloperoxidase					
MCC	1,68±0,01	1,32±0,01	1,32±0,02	0,67±0,07	0,81±0,02
P		<0,001	<0,001		>0,1
PLC, %	99,0±0,6	85,8±1,6	82,6±3,3	52,4±9,6	53,3±2,2
P		<0,001	<0,001		>0,5
Acid phosphatase					
MCC	0,65±0,01	0,49±0,01	0,25±0,01	0,76±0,03	0,8±0,01
P		<0,001	<0,001		>0,5
PLC, %	63,0±1,8	46,6±1,6	23,0±1,5	75,5±3,4	75,0±1,4
P		<0,001	<0,001		>0,5

Legend. PAL) Phagocytic activity of leukocytes, IC) ingestive capacity, ICP) index of completion of phagocytosis, MCC) mean cytochemical coefficient, PLC) positively labeled cells. Significance of differences between values for experimental animals determined by comparison with corresponding (depending on time of year) control.

neutrophils after 1 month and recovery of its reserve potential by the 3rd and 6th months of investigation. Weakening of the leukocytic response to injection of hydrocortisone compared with the control is evidence of marked limitation of the granulocytic reserve of the bone marrow 1, 3, and 6 months after administration of the cytostatic.

The response of the erythroid branch of the bone marrow to blood loss 1, 3, and 6 months after administration of rubomycin was characterized by a decrease in the rate of proliferation of the erythroid cells compared with the control, as shown by the lower ($0.001 < P < 0.01$) values of the number of stathmokinetic mitoses in the erythronormoblasts in the experimental animals at all times of observation. Meanwhile analysis of the trend of the absolute number of erythroid cells in the first 2 weeks after blood loss did not reveal any statistically significant difference between the values of this parameter in the groups compared.

Thus dispite a sufficiently large number of erythroid tissue cells capable of reproduction, the potential capacity of the proliferative pool of the rythron in the late stages after injection of rubomycin remained restricted.

It can be concluded from analysis of the data that a course of injections of rubomycin in a therapeutic dose causes marked limitation of the reserve capacity of hematopoiesis in rats, which cannot be detected by the ordinary methods of investigation of cellular homeostasis, but which is manifested after 1, 3, and 6 months by inhibition of the compensatory reactions of granulocytopoiesis and erythropoiesis in models in which hematopoietic function is subjected to stress.

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