PHARMACOLOGY AND TOXICOLOGY

Effects of Exogenous Glucocorticoids on Colony-Forming Activity of the Bone Marrow Under Cytotoxic Exposure

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Experiments on CBA/CaLac mice receiving a half-maximum tolerated dose of 5-fluorouracil demonstrated an inhibitory effect of physiological concentrations of dexamethasone (127×10^{-9} M) on colony-forming activity of erythroid and granulomonocytic precursors in intact and regenerating bone marrow. Dexamethasone exhibited protective effects on granulocyte, macrophage, and fibroblast precursors during maximum myelosuppression. Various responses of hemopoietic progenitor cells to exogenous corticosteroid *in vitro* are probably determined by their functional state.

Key Words: *dexamethasone; hemopoietic precursors; 5-fluorouracil*

The ability of glucocorticoids to affect directly (through receptors) and indirectly (through hemopoiesis-inducing microenvironmental cells) proliferation and differentiation of committed hemopoietic progenitor cells is beyond doubt. However, there is no general agreement regarding their controlling effects on the growth of myeloid progenitor cells in culture. There are data on both stimulatory [6,9] and inhibitory [6-8] effects of dexamethasone and other natural and synthetic corticosteroids on proliferation of erythroid (CFU-E) and granulomonocyte (CFU-GM) colony-forming units. The ambiguity of these results is due to various experimental conditions and pharmacological concentrations of the preparations used.

The response of hemopoietic cells to exogenous corticosteroids is probably determined by their functional state. Therefore, this work was designed to study *in vitro* effects of physiological concentrations of dexamethasone on bone marrow colony-forming capacity during myelosuppression induced by 5-fluorouracil (5-FU).

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MATERIALS AND METHODS

Experiments were performed on 35 male CBA/CaLac mice weighing 18-20 g (Laboratory of Experimental Biomedical Modelling, Tomsk Research Center).

The mice were intraperitoneally injected with 114 mg/kg 5-FU in a half-maximum tolerated dose for modeling myelosuppression. Bone marrow nuclears from the femur (10^7 cells) were cultured for 1 h with 127×10^{-9} M dexamethasone (Hafslund Nycomed Pharma) [5] in 1 ml RPMI-1640 (Sigma) containing 5% fetal bovine serum (Sigma), 10 mM HEPES (Flow), 2 mM L-glutamine (Sigma), and 40 mg/liter gentamicin. Myelokaryocytes were washed twice with the medium and cultured (3×10^5 viable cells/ml) in methyl cellulose [2] for determining the concentrations of CFU-E, CFU-GM, and stromal fibroblast precursor cells (CFU-F). Human recombinant erythropoietin (0.5 U/ml, Sigma) served as the growth stimulator for CFU-E. CFU-GM and CFU-F were cloned in the presence

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TABLE 1. Concentrations of Hemopoietic Precursors (10^5 Myelokaryocytes) in the Bone Marrow of Mice Injected with 5-FU and Dexamethasone after Stimulation with Growth Factors ($X \pm m$)

Experiment	CFU-E		CFU-GM		CFU-F	
	5-FU	5-FU+dexamethasone	5-FU	5-FU+dexamethasone	5-FU	5-FU+dexamethasone
Control	13.18±0.63	7.78±0.73+	4.08±0.95	1.79±0.44+	12.88±0.95	11.67±0.58
1 h	2.78±0.43*	3.62±0.45	1.80±0.45*	2.63±0.73	0.70±0.30*	1.67±0.32+
Day 3	9.30±0.88*	9.03±0.85	3.75±0.58	7.58±0.80+	4.58±0.73*	12.27±0.95+
Day 5	11.53±1.03	5.13±0.58+	15.70±1.48*	8.33±0.89+	—	—

Note. Here and in Table 2: $p<0.05$: *compared with the control, +compared with 5-FU.

of 15% supernatant of splenocytes stimulated with 15 μg/ml phytohemagglutinin (Serva) alone or in combination with 4×10^{-9} g/ml recombinant mouse GM colony-stimulating factor (Sigma). The number of colonies was determined in 96-well flat-bottom plastic plates (Corning) on the 3rd and 7th days in culture, respectively.

The data were analyzed statistically using Student's *t* test.

RESULTS

The concentration of committed hemopoietic progenitor cells in the bone marrow decreased significantly 1 h after the administration of 5-FU. The concentrations of CFU-GM, CFU-E, and CFU-F decreased to 65%, 21%, and 5% of their initial levels, respectively (Tables 1 and 2). The contents of CFU-E and CFU-GM were restored on the 5th and 3rd days, respectively. On the 5th day, the number of CFU-GM 4-fold surpassed the initial level. The concentration of CFU-F in the bone marrow hemopoietic tissue decreased to 36% on the 3rd day and was restored on the 5th day. This was confirmed by dense fibroblast growth forming a stromal layer for CFU-GM. These findings are consistent with the data on toxic effects of the 5-FU on committed hemopoietic progenitor cells [1,3].

Effects of dexamethasone on the colony-forming capacity of the bone marrow varied and depended on the functional state of hemopoietic progenitor cells. Preincubation of myelokaryocytes from intact mice with dexamethasone for 1 h sharply decreased the yields of CFU-E and CFU-GM in the bone marrow methyl cellulose culture (Table 1). This hormone inhibited the formation of erythroid bursts during culturing of embryonic liver cells in plasma clot [7]. The concentration of CFU-F did not change significantly (Table 1).

Dexamethasone had no considerable effect on the growth of erythroid colonies in unfractionated bone marrow culture taken 1 h and 3 days after administration of 5-FU, but led to a twofold decrease in the concentration of CFU-E on the 5th day. Similar inhibition of the formation of CFU-GM was observed *in vitro*.

Preincubation with dexamethasone increased (by 2-3 times) the concentrations of CFU-GM and CFU-F 1 h after and to a greater extent on the 3rd day after 5-FU injection (Table 1). The concentration of CFU-F exceeded its initial level on the 3rd day.

Thus, dexamethasone administered in the same dose can inhibit or stimulate or produced no considerable effect on the colony-forming capacity of hemopoietic progenitor cells *in vitro*. This may be due to different functional states of these cells in the dynamics of cytotoxic effect of 5-FU. Indeed, CFU-E are known to be more sensitive to the cytostatic than CFU-GM [3,4]. Moreover, catecholamines that are closely associated with glucocorticoids can modulate *in vitro* and *in vivo* effects of 5-FU on the colony-forming and proliferative capacities of committed hemopoietic progenitor cells [4].

Hydrocortisone can protect blast cells from lethal effects of arabinosylcytosine [10]. Adrenoceptor antagonists administered on the 3rd day after injection of 5-FU increase the number of CFU-E and CFU-GM in the DNA synthesis phase [1]. Therefore, the dexamethasone-induced increase in the colony-forming capacity of bone marrow cells on the 3rd day after

TABLE 2. Concentrations of Hemopoietic Precursors (10^5 Myelokaryocytes) in the Bone Marrow of Mice Injected with 5-FU and Dexamethasone after Stimulation with Mouse Splenocyte Supernatant ($X \pm m$)

Experiment	CFU-GM	
	5-FU	5-FU+dexamethasone
Control	3.12±0.45	1.60±0.25+
Day 3	3.13±0.67	3.33±0.68
Day 5	5.63±0.87*	2.85±0.85+

administration of 5-FU may be associated with its protective effects on cytostatic-damaged CFU-GM and CFU-F. Protective action of the glucocorticoid is probably mediated by fibroblasts, because this effect was not observed in the absence of CFU-F growth in culture containing no GM colony-stimulating factor (Table 2). However, the inhibitory effects of dexamethasone on CFU-E and CFU-GM in intact mice and in mice administered with 5-FU on the 5th day postinjection (the initial period of postcytostatic proliferation of the hemopoietic tissue) are beyond question (Tables 1 and 2) [3].

Our findings demonstrate suppressive effects of physiological concentrations of dexamethasone on the colony-forming capacity of intact and regenerating bone marrows. However, this synthetic glucocorticoid protects CFU-GM and CFU-F during maximal myelosuppression induced by the cytostatic. These protective effects are probably mediated by cooperation of stromal and hemopoietic cells.

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